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# STUDIES OF DIABETES MELLITUS

## I. RESPIRATORY EXCHANGE FOLLOWING THE INGESTION OF GLUCOSE, GLYCEROL, CALCIUM HEXOSE PHOSPHATE AND CALCIUM GLYCEROPHOSPHATE

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Many studies have been made of the respiratory exchange in diabetes mellitus, which have thrown a great deal of light on the nature of the disease and upon the mechanism of carbohydrate metabolism in general. More recently with the advent of rapid analytical methods for the determination of the chemical constituents of blood, attention has been focused on the blood sugar and its regulation, and studies of the respiratory exchange have become relatively uncommon. It is believed that a revival of interest in the study of the respiratory exchange in diabetes will yield information of importance in understanding the disease, especially when such studies are made in conjunction with careful clinical and chemical observations.

In the present study a comparison has been made of the effects on the respiratory exchange of the ingestion of glucose and of the calcium hexose phosphoric ester. A similar comparison has been made with glycerol and glycerophosphates of calcium and sodium. Interest in such a comparison was stimulated by the apparent importance of phosphates for carbohydrate metabolism in general as shown in the work of Harden and Young,[1] who demon-

[1] Harden: Alcoholic Fermentation. Biochemical Monographs. Longmans, Green and Co., London.

strated that the formation of a hexose-diphosphoric ester was an essential factor in the fermentation of sugars by yeast. This discovery becomes doubly significant in view of the demonstration of a similar substance, having the same elementary composition and yielding the same osazone, in mammalian muscles in which it is the probable precursor of lactic acid. The literature on this subject has become voluminous. It will be sufficient to refer chiefly to the work of Euler, Embden, Laquer, and their coworkers.[2,3,4,5]

It will be seen that there is evidence that the carbohydrate formed by hydrolysis of the hexose phosphate is fructose.

Von Lebedew and Griaznoff[6] believe that dextrose is first split to dioxyacetone and glyceric aldehyde, that dioxyacetone forms an ester with phosphoric acid and that glyceric aldehyde does not. The elementary analysis would correspond either with a compound of the triose combined with one molecule of phosphoric acid or of the hexose combined with two molecules. The molecular weight of the compound has not yet been determined.

### Methods and Apparatus

Patients were sent to the laboratory in the morning in a wheel chair, without breakfast. The wheel chair was arranged so that the patient or normal subject remained in a semi-reclining posture for 30 minutes at least before the beginning of the experiment. An observation of the basal respiratory exchange was made in each case. Following this the patient was given sugar, glycerol, or phosphoric esters in a cup of Kaffee Hag. Urine specimens were collected and the time of voidings carefully noted. The time intervals covered by the specimens of urine were so arranged that the respiratory observations occurred at about the middle of the interval. From the total nitrogen figures the rate of nitrogen excretion per hour was determined. The respiratory exchange was observed after an interval of 40—50 minutes and again from 100 to 140 minutes following the ingestion of the various substances.

In the experiments on W.A., and on Samuel P., a French army gas mask was used with valves obtained from the Sanborn Co. In all subsequent experiments the expired air was separated from the inspired by means of a Lovén valve, the subject's nose being closed with a clip. The expired air was collected in an accurately counterpoised spirometer and measured, gas temperature and barometric pressure being noted. The samples of gas for analysis were collected in a special sampling bottle over a glycerine-salt solution. The carbon dioxide of the expired air stored for 24 hours in such a container diminishes by about 0.15%. Gas analyses were practically always made in less than six hours, a modified Henderson-Haldane apparatus being used. Duplicate analyses were made and accepted if the agreement was within 0.04% for both oxygen and carbon dioxide. From these data the indirect calorimetry was calculated by the method of Zuntz and Schumburg.[7]

One may question the reliability of respiratory quotients obtained in experiments in which either a mask or mouthpiece is used to connect the patient with the respiratory valves. Many subjects are entirely unsuitable for study in any other apparatus than a respiration chamber. Success in securing reliable quotients depends upon careful observation of the following points:—the comfort of the patient as regards position and adjustment of mouthpiece and nose-clip, absence of a large dead space, absence of resistance to the opening of the valve membranes, accurate counterpoising of the spirometer, and absence of sound in the valve. These points have been carefully attended to. After a trial of many forms of valves and masks it is the opinion of the writers that the Lovén valve used with a mouthpiece is the most satisfactory one obtainable. A description of this valve will be found in a previous publication by one of us.[8]

Careful observation of the type of respiration and its rate was made before and after the adjustment of the mouthpiece and nose-clip. A sufficient time was allowed for the respiration to assume its normal character before the collection of the expired air was commenced. In all of the subjects of the experiments here recorded there was so little disturbance of the respiration that the respiratory quotients are believed to approach the actual metabolic quotients very closely.

The calcium hexose phosphate used in these experiments was obtained through the kindness of Mr. Raymond Foster of the Bayer Company, in whose factories it was prepared from yeast fermentation of dextrose. The trade name of this substance is candiolin. Analyses of this substance show that it corresponds fairly closely with the empirical formula $C_6 H_{10} O_4 (PO_4)_2 Ca$. It contains a small amount of moisture, small amounts of inorganic phosphate, and very small amounts of free carbohydrate. After hydrolysis it became levo-rotary.

### Discussion of Results

The data of the respiration experiments are recorded in Table 1, comprising six diabetic subjects and seven normal men. A full report of these cases, giving all the details of metabolic studies and of the clinical course under

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[2] Euler: Verhalten der Kohlenhydratphosphorsauere-ester im Tierkoerper. Zeitschr. f. physiol. Chem., 1912, LXXIX, 375.

[3] Embden and Laquer: *ibid.*, 1921, CXIII, 1.

[4] Embden and Adler: *ibid.*, 1921, CXIII, 201.

[5] Laquer, F.: *ibid.*, 1921, CXVI.

[6] von Lebedew and Griaznoff: Bericht. Deutsch. chem. Gesellsch., 1912, XLV, 3256.

[7] Zuntz u. Schumburg: Studien zu einer Physiologie des Marsches. Berlin, 1901, p. 361. See also Lusk, G., Science of Nutrition. 3rd Ed., 1917, p. 62. Saunders and Co., Philadelphia.

[8] McCann: The effect of the ingestion of foodstuffs on the respiratory exchange in pulmonary tuberculosis. Arch. Int. Med., 1921, XXVIII, 847.

treatment, will be made in the second paper of this series. The patients were treated by restriction to a maintenance diet, low in protein, and balanced as to ketogenic and antiketogenic factors, until glycosuria and hyperglycemia were relieved.

(a). *The effect of the ingestion of glucose* upon the respiratory exchange of many diabetics, during periods in which either glycosuria, hyperglycemia, or both were present, is markedly different from that produced in normal subjects. A large number of observations on normal individuals may be found in the literature, the most comparable for our purposes being those of Benedict and Carpenter,[9] and of Higgins.[10] For this reason we have included only a small number of normal experiments of our own, as these correspond with the usual findings. Normally, following the ingestion of sugars, the respiratory quotient begins to rise within 9—15 minutes as a rule. In some experiments a slight decrease in quotient may be observed within this time. It is very rarely that the respiratory quotient fails to rise within 30 minutes after sugar ingestion in normal subjects in normal nutritive condition. In normal individuals, under circumstances which tend to exhaust the stores of glycogen, such as hard work on a high protein-low carbohydrate diet, Bernstein and Falta [11] found that the ingestion of carbohydrate is not followed by the usual rise in carbon dioxide until the glycogen stores have been replenished.

The subject W.A., was given glucose on three occasions. On the 29th of January, 1922, he showed glycosuria and hyperglycemia. On this day his respiratory quotient was definitely lower than the basal quotient 50 minutes after the ingestion of sugar; though it was found to have risen at the end of two hours. The test was not repeated when the patient had become sugar-free and when his tolerance had increased. However, he returned to the clinic in November 1922, again showing marked glycosuria. On the 11th of November his basal respiratory quotient was very low, below 0.70. The ingestion of glucose caused a further marked depression of the quotient which was still present at the end of an hour and a half. A final observation was made on this patient, after glycosuria had disappeared and an increase of more than 100 grams in carbohydrate utilization had occurred, on December 22, 1922. On this last occasion no fall in the respiratory quotient was observed; instead a slight rise occurred.

In the case of the subject Samuel P., glucose was given on three occasions, on all of which glycosuria was noted, though the fasting blood sugar level was not high prior to the test. Glucose given alone, with tricalcium phosphate, and with hexose phosphate, caused a fall in the value of the respiratory quotient obtained about 40 minutes after ingestion. The quotient at the end of two hours was elevated above the basal value on each occasion. A similar type of change in quotients was found in the case of Ostella B.

The subject Charles G., was given glucose on April 21, 1922, at which time he had a marked glycosuria and hyperglycemia. The respiratory quotients decreased progressively during an observation of two hours after ingestion. On the 26th of May he was given fructose and on the 29th glucose, at a time when his carbohydrate utilization had increased more than 150 grams above that of April 21. On both of these latter tests the quotients were found to be elevated at the end of 45—50 minutes after ingestion, and in both cases it was lower at the end of two hours.

Two remaining subjects, Lorena T., and Howard M., both severely diabetic, show an entirely different behavior of the respiratory quotients after the ingestion of glucose. In spite of the fact that these subjects had marked glycosuria and hyperglycemia, and that the diabetes could not be controlled by restriction of the diet to the basal requirement, the rise in respiratory quotients which followed the ingestion of sugar corresponded quite well with that which we have observed in normal subjects.

There appear, therefore, to be two types of change produced in the respiratory exchange after the ingestion of glucose by individuals who are in the stage of severe diabetes with glycosuria and hyperglycemia. In one type the respiratory quotients are depressed for an abnormal length of time. In the other type the quotients rise at a rate approximating normal. Of the individuals of the former type all were responsive to treatment and subsequently a good carbohydrate utilization. Of the two subjects of the latter type both failed entirely to respond to treatment of any kind. That the clinical improvement of the patients of the first type is accompanied by an increased ability to oxidize glucose is shown in the cases of W.A., and of Charles G.

Interesting questions arise concerning the significance of these two different types of response to glucose ingestion. One of the first which arises is whether there may not be two (or more) types of diabetes mellitus at present clinically indistinguishable, but in which the disorder involves different mechanisms. That animals deprived of the pancreas show a diminished rate of oxidation of glucose is well known.[12] Conversely, that the injection of the pancreatic extract, "insulin," of Banting,[13] increases the respiratory quotient and rate of oxidation of sugar is also known. What then is the mechanism at fault in a

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[9] Benedict, F. G., and Carpenter, T. M.: Food Ingestion and Energy Transformations. Publication 261, p. 206-208, Carnegie Inst. of Washington.

[10] Higgins, H. L.: The rapidity with which alcohol and some sugars may serve as nutriment. Am. Journ. Physiol., 1916, XLI, 258.

[11] Bernstein and Falta: Respiratorischer Stoffwechsel und Blutzuckerregulation. Deut. Arch. f. klin. Med., 1918, CXXV, 233.

[12] Verzar: Biochem. Zeitschr., 1914, LXVI, 75.

[13] Banting, Best, Collip, Hepburn, Macleod, and Noble: The physiological effects of Insulin. Trans. Royal Soc. Can., 16, Sect. V, 1-18, 1922.

type of severe diabetes in which there is an apparent ability to oxidize carbohydrate at a fairly normal rate? This question cannot be answered with the data here available. Much light may be shed upon it by experiments now in progress on the effects of the pancreatic extract "insulin," which will be reported in a later communication.

A recent paper by Achard and Binet [14] bears on the observed occurrence of a fall in respiratory quotients in diabetic subjects following the ingestion of sugar. Their brief report does not give experimental data, but in their experiments, in which expired air was collected in a Tissot spirometer and analysed only for $CO_2$, they found that after the ingestion of sugar both $CO_2$ production and the minute volume of respired air diminished. This is undoubtedly the phenomenon which has been observed in the present study. Its true significance can not have been apparent to Achard and Binet because of their failure to determine simultaneously the oxygen consumption.

In some of the experiments the so-called "diabetic respiratory quotient" was obtained. This refers to quotients less than 0.70. An explanation of such respiratory quotients has been given by Lusk,[15] who calculated the theoretical and observed quotients in the case of a complete phlorhizin glycosuria and in the case of a completely diabetic man. The calculation is made of the effect upon the respiratory quotient of protein produced by an entire failure to oxidize any of the dextrose arising from the amino acids of the protein. Under these circumstances, when the D:N ratio is 3.65, the respiratory quotient of protein would be 0.632. Lusk also makes use of Magnus Levy's [16] calculation of the maximum formation of beta-oxybutyric acid from fat to explain low respiratory quotients (100 gm. fat yield a maximum of 36 gm. beta-oxybutyric acid). The effect of beta-oxybutyric acid formation upon the respiratory quotient of fat would be to lower the R.Q. from 0.707 to 0.669. However, enough carbon dioxide might be liberated from bicarbonates to increase this somewhat, the amount depending on the extent to which the beta-oxybutyric acid combined with other substances.

One of us has observed [17a] such a diabetic quotient in the course of an experiment on a non-diabetic man, who ingested a meal of meat at the end of a fast of eight days. The explanation offered in this case was that the fasting had so depleted the glycogen reserves of the subject that the sugar formed in the metabolism of the protein ingested was stored as glycogen and not oxidized at once.

In a study of a patient with severe diabetes Wilder, Boothby and Beeler [17b] found depression of the respiratory quotients after meals rich in protein, but containing carbohydrate and fat.

The question as to why the rate of oxidation of carbohydrate is diminished in some cases after sugar ingestion is a very difficult one. If the ingested sugar were to pass first into the depleted storehouses of glycogen, as is suggested by the work of Bernstein and Falta,[11] it is difficult to understand why this should have the inhibiting effect which has been observed upon the oxidation of carbohydrate already in the metabolism.

It is of interest to know whether or not glucose exhibits a specific dynamic action in diabetic subjects. The experiments show considerable variation. In two diabetic patients a decrease in heat production was noted, even in periods in which the respiratory quotient was increasing. One of these subjects (W.A.) showed an increase of 20% in metabolism at a time when the R.Q. was falling. In other subjects the increase in metabolism varied from 7 to 14 per cent irrespective of the direction of change in R.Q. Of the three normal subjects one showed no increase in metabolism and the other two showed increases of 7% and 10% respectively.

(b). *Experiments with glycerol.* Two experiments are given to show the effects of glycerol on the respiratory exchange of diabetic subjects; these are the experiment on W.A., of Feb. 8, 1922, and that on Charles G., May 3, 1922. In both cases the changes in respiratory quotient ran parallel to those produced by glucose in the same subjects. In the case of W.A., the basal respiratory quotient was 0.821; 40 minutes later it was 0.759, 2 hours and 15 minutes after the ingestion of glycerol it was 0.840. The same depression of the value of the quotient, with a subsequent rise, was noted when glucose was given to the same subject on January 29th. The experiments with Charles G. show a similar parallelism, though in this case the quotients following the ingestion of both glycerol and glucose continued to diminish during the whole period of observation of a little over two hours.

Three experiments were made upon normal subjects to whom glycerol was given, W.A.P., R.S.L., and R.J.B. In the case of W.A.P. there proves to be no parallelism between the effects produced by glucose and by glycerol. With glucose a marked rise in quotient was observed, but with glycerol the quotient was diminished during a period of an hour and a half after ingestion. A similar diminution of the quotient was observed in the case of R.S.L. With the subject R.J.B. a transient rise of the quotient was noted, followed by a fall.

The specific dynamic action of glycerol in the three normal subjects varied from zero to 4.0 per cent. In one of the two diabetic subjects no significant change in the

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[14] Achard et Binet: Compt. rend. Soc. biol., 1922, LXXXVII, 52.

[15] Lusk, Graham: Clinical Calorimetry, Eighth Paper. On the diabetic respiratory quotient. Arch. Int. Med., 1915, XV, 939.

[16] Magnus Levy: Zeitschr. f. klin. Med., 1905, LVI, 83.

[17a] McCann: An observation of the effect of a protein meal given to a man at the end of an eight day fast. Proc. Soc. Exper. Biol. Med., 1920, XVII, 173.

[17b] Wilder, R. M., Boothy, W. M., and Beeler, C.: J. Biol. Chem., 1922, LI, 311.

heat production occurred. In the other a rise of 20% in metabolism was noted in an experiment in which the values of the R.Q. were decreased by the ingestion of glycerol.

That glycerol is convertible into glucose was shown by Cremer,[18] who administered glycerol to dogs under the influence of phlorhizin. When the D:N ratio was complete (3.65) the extra glucose arising from glycerol was about 35—40 per cent of the weight of the glycerol.

It is impossible to calculate the oxidation of the glycerol in these experiments. The calculation of Zuntz and Schumburg [7] has been made, however, just as though the non-protein respiratory quotient were determined only by fat and carbohydrate, the figures in the table being in brackets. The respiratory quotient for glycerol should be approximately 0.86.

(c). *Experiments with hexose phosphate.* A comparison of the effects of ingesting calcium hexose phosphate with those produced by glucose is possible in the cases of the diabetic subjects W.A., Samuel P., Lorena T., and Charles G., and of the normal subject R.R.H. In the experiments on W.A., a progressive rise in quotients followed the ingestion of the hexose phosphate in contrast to the initial depression of the quotient produced by the ingestion of glucose. In those on Samuel P. a similar contrast of behavior of the respiratory quotients was noted. A progressive rise of quotients followed the taking of hexose phosphate, a depression of quotients being produced by glucose, which was also observed when glucose was taken together with tricalcium phosphate and with hexose phosphate. Calcium phosphate taken alone did not affect the respiratory quotient.

In the experiments on Charles G., glucose ingestion produced a fall in value of the respiratory quotient from 0.73 to 0.69. In this case the taking of hexose phosphate caused a transient decrease in quotient from 0.73 to 0.71 with a subsequent rise to 0.77.

In the experiments on the diabetic subject Lorena T. and on the normal R.R.H., a steady rise in R.Q. values followed the ingestion of both glucose and hexose phosphate.

These experiments indicate that hexose taken in the form of an ester of phosphoric acid is more readily oxidized in certain diabetics than is glucose. Practical advantage can not be taken of this fact, because of the physical impossibility of ingesting a sufficient quantity of sugar in this form, unless it can be shown that the assimilation of a large amount of phosphates can influence favorably the utilization of carbohydrates in general. There is nothing in the experiments presented here which would indicate such an effect.

The heat production was increased following the ingestion of calcium hexose phosphate in all experiments. The rise in metabolism varied between 4.5 and 12 per cent.

[18] Cremer: Muench. med. Wochenschr., 1902, XLIX, 944.

(d). *The effect of the ingestion of glycerophosphates* shows considerable variation. With the diabetic subjects W.A., and Samuel P., the respiratory quotients rose. With the normal subject W.S.M. and with the diabetic subjects Lorena T. and Charles G. there was observed to be an initial drop in quotient followed by a rise. The calcium salt was ingested in all cases except that of Lorena T., to whom an intravenous injection of sodium glycerophosphate was given.

The effect upon the heat production was to cause a slight fall in three experiments, and a rise of 5.5 and 8.7 per cent respectively in the other two.

Only one normal subject was able to retain calcium glycerophosphate. In two others violent vomiting ensued. Nausea was quite marked in the case of the normal subject, but was complained of less by the diabetic patients.

## Summary

1. In a study of the effects of glucose ingestion on the respiratory exchange of diabetic subjects two types of response were encountered. In the first type of subject, ingestion of glucose resulted in a diminished rate of carbohydrate oxidation, as shown by a decrease in the respiratory quotients for an abnormal length of time. These subjects all improved greatly under treatment with the development of a good carbohydrate tolerance, showing on subsequent tests an improved ability to oxidize glucose. A second type of patient with severe diabetes was encountered in whom the ingestion of glucose produced a rise of respiratory quotients at a rate similar to that of normal subjects. These individuals were not responsive to treatment with a maintenance diet, low in protein, and balanced as regards ketogenic and antiketogenic factors.

A rise in heat production may or may not follow the ingestion of glucose by diabetic subjects. It occurred in both subjects of the second type. In the first type the heat production may increase with falling respiratory quotients and vice versa.

2. The changes in respiratory quotients of diabetic subjects who ingested glycerol were parallel to those produced by the ingestion of glucose in the same subjects. In normal subjects there was no parallelism between the effects of glycerol and glucose. The specific dynamic action of glycerol was negligible in all but one experiment. In this one case an increase in total metabolism of 20% was observed coincidently with a decrease in R.Q.

3. A comparison of the effects of ingestion of calcium hexose phosphate with those produced by glucose showed that in subjects of the first type hexose from the phosphoric ester was oxidized more readily than glucose. Hexose phosphate ingestion caused a steady rise in respiratory quotients in both diabetic subjects and in a normal individual. It was followed by a rise in heat production of from 4.5—12 per cent in all experiments.

TABLE I. Data of Respiration Experiments.

| Date 1922 | Name Area Sq. M. Age Sex | Time | Vol. expired L. per Hr. S.T.P.D. | $CO_2$ Per cent produced | $O_2$ Per cent absorbed | R. Q. | Urine N. per hr. Gm. | Non Protein R. Q. | Calories per hour from Carbo. | Fat | Prot. | Total | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-25 | W. A. | 9:21- 9:31 | 332.4 | 2.71 | 3.51 | 0.770 | .216 | 0.761 | 10.2 | 39.0 | 5.7 | 54.9 | Basal. 92% average normal. |
| | 1.51 Sq. M. | 9:32- 9:40 | | | | | | | | | | | Ingested 60 Gm. Hexose Phosphate. |
| | 22 | 10:26-10:32 | 368.6 | 2.65 | 3.39 | 0.782 | .177 | 0.780 | 13.7 | 41.0 | 4.7 | 59.4 | 50′ after ingestion. |
| | Diabetic | 11:45-11:51 | 372.9 | 2.72 | 3.35 | 0.812 | .217 | 0.813 | 19.7 | 34.4 | 5.7 | 59.8 | 129′ after ingestion. |
| 1-29 | W. A. | 9:41- 9:47 | 327.4 | 3.04 | 4.04 | 0.752 | .251 | 0.746 | 7.5 | 48.1 | 6.6 | 62.2 | Not basal. Coffee at 8:00 A.M. |
| | 1.51 Sq. M. | 9:52 | | | | | | | | | | | Ingested 37.5 Gm. Glucose. |
| | 22 | 10:42-10:48 | 343.8 | 2.85 | 3.89 | 0.732 | .253 | 0.723 | 3.2 | 52.6 | 6.7 | 62.5 | 50′ after ingestion. |
| | Diabetic | 12:01-12:07 | 343.7 | 2.87 | 3.39 | 0.846 | .320 | 0.855 | 24.1 | 23.4 | 8.5 | 56.0 | 122′ after ingestion. |
| 2-7 | W. A. | 9:56-10:02 | 309.4 | | | (0.764) | .263 | (0.756) | | | | | Basal. Gas. Sample lost R.Q. is average of 2. |
| | 1.51 Sq. M. | 10:05-10:25 | | | | | | | | | | | Ingested 100 Gm. Calcium Glycerophosphate. |
| | 22 | 10:48-10:54 | 299.9 | 3.11 | 4.07 | 0.764 | .213 | 0.759 | (9.4) | (42.6) | (5.6) | 57.6 | 33′ after ingestion. |
| | Diabetic | 11:25-11:31 | 314.8 | 3.00 | 3.82 | 0.785 | .149 | 0.784 | (14.5) | (38.8) | (4.0) | 57.3 | 70′ after ingestion. |
| | | 12:06-12:12 | 298.0 | 3.22 | 4.08 | 0.789 | .149 | 0.788 | (15.1) | (38.9) | (4.0) | 58.0 | 111′ after ingestion. |
| 2-8 | W. A. | 9:16- 9:22 | 340.9 | 3.03 | 3.71 | 0.821 | .131 | 0.817 | (21.7) | (36.6) | (3.5) | 60.8 | Basal. 102% of average normal. |
| | 1.51 Sq. M. | 9:41- 9:45 | | | | | | | | | | | Ingested 40 Gm. Glycerol. |
| | 22 | 10:23-10:29 | 358.6 | 2.67 | 3.52 | 0.758 | .131 | 0.756 | (9.6) | (46.6) | (3.5) | 59.7 | 40′ after ingestion. |
| | Diabetic | 11:58-12:04 | 356.2 | 2.93 | 3.49 | 0.840 | .178 | 0.843 | (25.7) | (29.5) | (4.7) | 59.9 | 135′ after ingestion. |
| 11-10 | W. A. | 8:35- 8:42 | 262.7 | 3.12 | 4.46 | 0.700 | .114 | 0.693 | 0 | 51.8 | 3.0 | 54.8 | Basal. 96% of average normal. |
| | 1.44 Sq. M. | 9:00 | | | | | | | | | | | Ingested 30 Gm. Glucose. |
| | 22 | 9:51- 9:56 | 287.3 | 3.25 | 4.87 | 0.667 | .164 | 0.657 | 0 | 61.0 | 4.4 | 65.4 | 51 Min. after ingestion. |
| | Diabetic | 10:29-10:35 | 253.8 | 3.33 | 4.91 | 0.678 | .174 | 0.667 | 0 | 53.6 | 4.6 | 58.2 | 89 Min. after ingestion. |
| 12-22 | W. A. | 9:38- 9:44 | 302.4 | 3.48 | 4.59 | 0.758 | .165 | 0.754 | 10.0 | 51.2 | 4.4 | 65.6 | Basal. 112% of average normal. |
| | 1.48 Sq. M. | 9:56 | | | | | | | | | | | Ingested 30 Gm. Glucose. |
| | 22 | 10:32-10:39 | 319.1 | 3.54 | 4.63 | 0.765 | .364 | 0.758 | 10.6 | 49.3 | 9.7 | 69.6 | 36 Min. after ingestion. |
| | Diabetic | 11:24-11:31 | 310.9 | 3.38 | 4.40 | 0.768 | .364 | 0.762 | 10.5 | 44.3 | 9.7 | 64.5 | 88 Min. after ingestion. |
| 2-15 | Sam'l P. | 9:50- 9:56 | 389.9 | 2.84 | 3.79 | 0.749 | .358 | 0.740 | 6.7 | 53.1 | 9.5 | 69.3 | Basal. 97% average normal. |
| | 1.81 Sq. M. | 10:04 | | | | | | | | | | | Ingested 50 Gm. Glucose. |
| | 21 | 10:42-10:48 | 435.8 | 2.69 | 3.73 | 0.721 | .340 | 0.709 | 0 | 66.0 | 9.0 | 75.8 | 38 Min. after ingestion. |
| | Diabetic | 12:11-12:17 | 390.6 | 3.06 | 4.01 | 0.763 | .340 | 0.757 | 11.3 | 53.5 | 9.0 | 73.8 | 127 Min. after ingestion. |
| 2-19 | Sam'l P. | 10:06-10:13 | 456.3 | 2.66 | 3.21 | 0.829 | .414 | 0.833 | 25.5 | 33.5 | 11.0 | 70.0 | Basal. 98% of average normal. |
| | 1.81 Sq. M. | 10:15 | | | | | | | | | | | Ingested 50 Gm. Glucose plus 20 Gm. $Ca_3(PO_4)_2$ |
| | 21 | 10:56-11:02 | 376.2 | 2.85 | 3.98 | 0.716 | .473 | 0.692 | 0 | 57.3 | 12.5 | 69.8 | 41 Min. after ingestion. |
| | Diabetic | 12:18-12:24 | 430.5 | 2.77 | 3.97 | 0.698 | .473 | 0.663 | 0 | 66.7 | 12.5 | 79.2 | 123 Min. after ingestion. |
| 2-22 | Sam'l P. | 9:36- 9:44 | 430.4 | 2.44 | 3.16 | 0.772 | .341 | 0.766 | 11.1 | 44.0 | 9.0 | 64.1 | Basal. 90% of average normal. |
| | 1.81 Sq. M. | 9:50 | | | | | | | | | | | Ingested 60 Gm. Hexose Phosphate. |
| | 21 | 10:34-10:40 | 453.4 | 2.49 | 3.18 | 0.783 | .473 | 0.778 | 13.6 | 41.9 | 12.5 | 68.0 | 44 Min. after ingestion. |
| | Diabetic | 12:08-12:14 | 438.4 | 2.53 | 3.13 | 0.808 | .352 | 0.809 | 19.7 | 36.3 | 9.3 | 65.3 | 138 Min. after ingestion. |
| 2-24 | Sam'l P. | 9:21- 9:29 | 419.7 | 2.75 | 3.46 | 0.795 | .444 | 0.793 | 16.9 | 40.2 | 11.7 | 68.8 | Basal. 96% of average normal. |
| | 1.81 Sq. M. | 9:42 | | | | | | | | | | | Ing. 50 Gm. Gluc. plus 30 Gm. Hexose Phosphate. |
| | 21 | 10:21-10:27 | 422.4 | 2.81 | 3.80 | 0.739 | .400 | 0.729 | 5.1 | 59.4 | 10.6 | 75.1 | 39′ after ingestion. |
| | Diabetic | 11:46-11:52 | 470.1 | 2.62 | 3.18 | 0.824 | .428 | 0.828 | 24.9 | 35.1 | 11.4 | 71.4 | 124 Min. after ingestion. |
| 2-27 | Sam'l P. | 9:34- 9:42 | 429.1 | 2.68 | 3.47 | 0.772 | .461 | 0.765 | 12.0 | 45.9 | 12.2 | 70.1 | Basal. 98% of average normal. |
| | 1.81 Sq. M. | 9:52 | | | | | | | | | | | Ingested 50 Gm. Calcium Glycerophosphate. |
| | 21 | 10:46-10:52 | 378.0 | 2.95 | 3.67 | 0.804 | .243 | 0.803 | (19.8) | (48.0) | 6.4 | 66.2 | 54′ after ingestion. |
| | Diabetic | 12:05-12:11 | 428.1 | 2.83 | 3.60 | 0.786 | .243 | 0.782 | (16.8) | (47.9) | 6.4 | 71.1 | 133′ after ingestion. |
| 3-8 | Sam'l P. | 9:50- 9:58 | 432.1 | 2.65 | 3.21 | 0.826 | .318 | 0.829 | 24.8 | 34.5 | 8.4 | 67.7 | Basal. 95% of average normal. |
| | 1.81 Sq. M. | 10:10 | | | | | | | | | | | Ingested 30 Gm. $Ca_3(PO_4)_2$ |
| | 21 | 11:03-11:13 | 423.6 | 2.70 | 3.26 | 0.828 | .427 | 0.834 | 23.8 | 30.9 | 11.3 | 66.0 | 53 Min. after ingestion. |
| | Diabetic | 12:03-12:12 | 436.3 | 2.77 | 3.38 | 0.820 | .363 | 0.823 | 24.2 | 36.6 | 9.6 | 70.4 | 113 Min. after ingestion. |
| 3-22 | W. S. M. | 10:00-10:06 | 365.9 | 3.07 | 3.87 | 0.793 | .465 | 0.790 | 15.6 | 39.0 | 12.3 | 66.9 | Basal. 97% of average normal. |
| | 1.75 Sq. M. | 10:15 | | | | | | | | | | | Ingested 30 Gm. Glycerophosphate. |
| | 32 | 10:57-11:04 | 351.3 | 3.02 | 3.88 | 0.778 | .438 | 0.772 | (11.8) | (40.8) | 11.6 | 64.2 | 43 Min. after ingestion. Nauseated. |
| | Normal | 12:13-12:20 | 366.2 | 3.02 | 3.79 | 0.797 | .280? | 0.796 | (17.9) | (40.7) | 7.4 | 66.0 | 120 Min. after ingestion. Nauseated. |
| 3-1 | R. R. H. | 9:51- 9:57 | 340.4 | 3.32 | 4.11 | 0.808 | .649 | 0.809 | 17.2 | 31.8 | 17.2 | 66.2 | Basal. 93% of average normal. |
| | 1.80 Sq. M. | 10:00 | | | | | | | | | | | Ingested 50 Gm. Glucose. |
| | 31 | 10:42-10:48 | 390.1 | 3.13 | 3.86 | 0.811 | .615 | 0.813 | 20.0 | 35.0 | 16.3 | 71.3 | 42 Min. after ingestion. |
| | Normal | 12:06-12:12 | 355.5 | 3.46 | 3.99 | 0.867 | .473 | 0.883 | 33.6 | 22.2 | 12.5 | 70.3 | 126 Min. after ingestion. |
| 3-20 | R. R. H. | 9:29- 9:36 | 362.5 | 2.88 | 3.96 | 0.727 | .411 | 0.711 | 0 | 54.4 | 10.9 | 65.3 | Basal. 92% of average normal. |
| | 1.80 Sq. M. | 9:42 | | | | | | | | | | | Ingested 30 Gm. Hexose Phosphate. |
| | 31 | 10:27-10:34 | 385.0 | 3.14 | 4.02 | 0.781 | .511 | 0.780 | 14.9 | 44.6 | 13.5 | 73.0 | 45 Min. after ingestion. |
| | Normal | 11:31-11:38 | 413.7 | 2.77 | 3.49 | 0.794 | .453 | 0.791 | 16.3 | 40.0 | 12.0 | 68.3 | 109 Min. after ingestion. |
| 3-27 | Lorena T. | 10:14-10:22 | 336.8 | 2.64 | 3.33 | 0.793 | .365 | 0.790 | 12.4 | 31.0 | 9.7 | 53.1 | Basal. 99% of average normal. |
| | 1.35 Sq. M. | 10:29 | | | | | | | | | | | Ingested 50 Gm. Glucose. |
| | 16 | 11:15-11:22 | 419.8 | 2.51 | 2.87 | 0.875 | .322 | 0.888 | 30.8 | 19.0 | 8.5 | 58.3 | 46 Min. after ingestion. |
| | Diabetic | 12:14-12:22 | 381.8 | 2.71 | 2.99 | 0.906 | .285 | 0.924 | 35.8 | 12.4 | 7.6 | 55.8 | 105 Min. after ingestion. |

TABLE I.—(Cont.)

Data of Respiration Experiments.

| Date 1922 | Name Area Sq. M. Age Sex | Time | Vol. expired L. per Hr. S.T.P.D. | $CO_2$ Per cent produced | $O_2$ Per cent absorbed | R. Q. | Urine N. per hr. Gm. | Non Protein R. Q. | Calories per hour from Carbo. | Fat | Prot. | Total | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-3 | Lorena T. | 9:38- 9:45 | 288.6 | 2.55 | 3.46 | 0.737 | .360 | 0.718 | 1.5 | 35.4 | 9.4 | 46.3 | Basal. 87% of average normal. Temp. 96.8° F. |
| | 1.35 Sq. M. | 9:51 | | | | | | | | | | | Ingested 30 Gm. Hexose Phosphate. |
| | 16 | 10:48-10:55 | 288.8 | 2.61 | 3.49 | 0.748 | .280 | 0.737 | 4.2 | 35.6 | 7.4 | 47.2 | 57 Min. after ingestion. |
| | Diabetic | 11:50-11:57 | 300.4 | 2.71 | 3.40 | 0.797 | .287 | 0.796 | 12.5 | 28.3 | 7.6 | 48.4 | 119 Min. after ingestion. |
| 4-14 | Lorena T. | 9:46- 9:52 | 296.1 | 2.52 | 3.47 | 0.726 | .295 | 0.721 | 2.1 | 38.0 | 6.3 | 46.4 | Basal. 87% of average normal. |
| | 1.35 Sq. M. | 10:24-10:30 | | | | | | | | | | | Intravenous inj. of Sodium Glycerophosphate. |
| | 16 | | | | | | | | | | | | M/6.5, 200 cc. $P_h$=7.3 (6.7 Gm.). |
| | Diabetic | 11:19-11:25 | 275.5 | 2.50 | 3.63 | 0.689 | .260 | 0.693 | (0) | (39.7) | 6.9 | 46.6 | 55 Min. after injection. |
| | | 12:31-12:37 | 292.3 | 2.44 | 3.37 | 0.724 | .238 | 0.737 | (4.2) | (35.0) | 6.3 | 45.5 | 127 Min. after injection. |
| 4-21 | Chas. G. | 9:37- 9:44 | 284.5 | 2.02 | 2.85 | 0.709 | .209 | 0.721 | 1.6 | 30.1 | 5.5 | 37.2 | Basal. 75% of average normal. Temp. 96° F. |
| | 1.17 Sq. M. | 10:12 | | | | | | | | | | | Ingested 30 Gm. Glucose. |
| | 14 | 10:48-10:55 | 288.3 | 2.09 | 2.97 | 0.704 | .241 | 0.714 | 0.9 | 31.9 | 6.4 | 39.2 | 36 Min. after ingestion. |
| | Diabetic | 12:08-12:15 | 294.1 | 2.06 | 3.04 | 0.678 | .219 | 0.682 | 0 | 35.2 | 5.8 | 41.0 | 116 Min. after ingestion. |
| 5-3 | Chas. G. | 9:27- 9:34 | 229.4 | 2.29 | 3.09 | 0.741 | .194 | 0.729 | 2.3 | 27.0 | 5.1 | 34.4 | Basal. 68% of average normal. |
| | 1.17 Sq. M. | 9:54 | | | | | | | | | | | Ingested 80 Gm. Glycerol. |
| | 14 | 10:47-10:54 | 247.6 | 2.44 | 3.58 | 0.682 | .118 | 0.671 | (0) | (38.3) | 3.1 | 41.4 | 53 Min. after ingestion. |
| | Diabetic | 11:59-12:09 | 194.5 | 2.75 | 4.07 | 0.676 | .149 | 0.666 | (0) | (32.7) | 3.9 | 36.6 | 125 Min. after ingestion. |
| 5-8 | Chas. G. | 9:37- 9:44 | 198.4 | 2.70 | 3.59 | 0.752 | .152 | 0.744 | 4.3 | 25.1 | 4.0 | 33.4 | Basal. |
| | | 10:10 | | | | | | | | | | | Ingested 30 Gm. Calcium Glycerophosphate. |
| | | 10:51-10:58 | 219.3 | 2.52 | 3.55 | 0.710 | .128 | 0.700 | (0) | (32.9) | 3.4 | 36.3 | 41 Min. after ingestion. |
| | | 11:50-11:57 | 222.5 | 2.56 | 3.32 | 0.771 | .082 | 0.769 | (7.1) | (25.8) | 2.2 | 35.1 | 100 Min. after ingestion. |
| 5-19 | Chas. G. | 9:58-10:04 | 212.3 | 2.48 | 3.25 | 0.763 | .236 | 0.730 | 2.1 | 23.8 | 6.3 | 32.2 | Basal. |
| | | 10:15 | | | | | | | | | | | Ingested 30 Gm. Hexose Phosphate. |
| | | 10:55-11:01 | 222.5 | 2.48 | 3.44 | 0.721 | .149 | 0.711 | 0 | 31.8 | 4.0 | 35.8 | 40 Min. after ingestion. |
| | | 12:23-12:29 | 233.3 | 2.53 | 3.25 | 0.779 | .159 | 0.774 | 7.3 | 24.4 | 4.2 | 35.9 | 128 Min. after ingestion. |
| 5-26 | Chas. G. | 9:37- 9:44 | 223.8 | 2.47 | 3.04 | 0.812 | .131 | 0.814 | 10.4 | 18.6 | 3.5 | 32.5 | Basal. |
| | | 10:25 | | | | | | | | | | | Ingested 30 Gm. Fructose. |
| | | 11:14-11:22 | 268.8 | 2.54 | 2.81 | 0.904 | .105 | 0.913 | 20.4 | 13.8 | 2.8 | 37.0 | 49 Min. after ingestion. |
| | | 12:20-12:27 | 244.0 | 2.51 | 3.16 | 0.794 | .108 | 0.793 | 10.0 | 23.9 | 2.9 | 36.8 | 115 Min. after ingestion. |
| 5-29 | Chas. G. | 9:49- 9:56 | 231.2 | 2.49 | 3.14 | 0.793 | | | | | | | Basal. |
| | | 10:20 | | | | | | | | | | | Ingested 50 Gm. Glucose. |
| | | 11:06-11:13 | 237.6 | 2.63 | 3.19 | 0.824 | | | | | | | 46′ after ingestion. |
| | | 12:13-12:20 | 231.0 | 2.57 | 3.43 | 0.749 | | | | | | | 113′ after ingestion. |
| 4-5 | Ostella B. | 9:45- 9:51 | 293.5 | 2.71 | 3.67 | 0.738 | .349 | 0.723 | 2.4 | 39.6 | 9.3 | 51.3 | Basal. 100% of average normal. |
| | 1.40 Sq. M. | 9:55 | | | | | | | | | | | Ingested 100 Gm. Glucose. |
| | 40 | 10:46-10:52 | 298.1 | 2.58 | 3.54 | 0.729 | .339 | 0.711 | 0 | 40.1 | 9.0 | 49.1 | 51′ after ingestion. |
| | Diabetic | 11:55-12:01 | 296.6 | 2.64 | 3.41 | 0.774 | .262 | 0.768 | 8.6 | 32.2 | 7.0 | 47.8 | 120′ after ingestion. |
| 9-5 | Howard M. | 10:33-10:41 | 257.8 | 2.33 | 3.44 | 0.677 | .152 | 0.663 | 0 | 37.4 | 4.0 | 41.4 | Basal. 84% of average normal. |
| | 1.15 Sq. M. | 10:47-10:51 | | | | | | | | | | | Ingested 30 Gm. Glucose. |
| | 16 | 11:33-11:41 | 302.5 | 2.37 | 3.14 | 0.754 | .185 | 0.748 | 5.7 | 34.2 | 4.9 | 44.8 | 42′ after Glucose. |
| | Diabetic | 12:41-12:49 | 313.6 | 2.26 | 2.81 | 0.804 | .156 | 0.804 | 12.7 | 25.2 | 4.1 | 42.0 | 110′ after Glucose ingestion. |
| 12-10 | R. S. L. | 9:24- 9:32 | 311.0 | 3.57 | 4.56 | 0.783 | .516 | 0.777 | 12.8 | 40.3 | 13.7 | 66.8 | Basal. 95% of average normal. |
| | 1.79 Sq. M. | 9:46 | | | | | | | | | | | Ingested 100 Gm. Glycerol. |
| | 31 | 10:28-10:36 | 316.7 | 3.45 | 4.61 | 0.747 | .394 | 0.737 | (6.2) | (51.8) | 10.4 | 68.4 | 42′ after ingestion. |
| | Normal | 11:23-11:31 | 301.0 | 3.65 | 4.88 | 0.748 | .549 | 0.731 | (4.6) | (49.3) | 14.6 | 68.5 | 97′ after ingestion. |
| 3-13 | W. A. P. | 9:41- 9:49 | 352.4 | 3.12 | 3.87 | 0.807 | .747 | 0.807 | 15.2 | 29.1 | 19.8 | 64.1 | Basal. |
| | 1.82 Sq. M. | 9:50 | | | | | | | | | | | Ingested 50 Gm. Glucose. |
| | 31 | 10:47-10:53 | 367.2 | 3.49 | 4.01 | 0.870 | .598 | 0.891 | 34.5 | 20.3 | 15.9 | 70.7 | 57 Min. after ingestion. |
| | Normal | 12:01-12:08 | 336.0 | 3.34 | 4.03 | 0.829 | .474 | 0.830 | 21.9 | 30.0 | 12.6 | 64.5 | 131 Min. after ingestion. |
| 12-12 | W. A. P. | 9:47- 9:55 | 371.7 | 3.29 | 3.90 | 0.844 | .508 | 0.854 | 28.2 | 27.8 | 13.5 | 69.5 | Basal. |
| | 1.8 Sq. M. | 9:59 | | | | | | | | | | | Ingested 100 Gm. Glycerol. |
| | 31 | 10:30-10:38 | | 3.25 | 3.99 | 0.815 | .656 | | | | | | 31 Min. after ingestion. |
| | Normal | 11:31-11:39 | 333.9 | 3.52 | 4.29 | 0.821 | .582 | 0.826 | (21.9) | (30.7) | 15.4 | 68.0 | 92 Min. after ingestion. |
| 12-13 | R. S. B. | 9:22- 9:28 | 368.0 | 3.75 | 5.03 | 0.745 | .538 | 0.733 | 6.5 | 64.2 | 14.3 | 85.0 | Basal. 112% of average normal. |
| | 1.96 Sq. M. | 9:55 | | | | | | | | | | | Ingested 100 Gm. Glycerol. |
| | 23 | 10:35-10:41 | 380.6 | 3.80 | 4.92 | 0.772 | .392 | 0.767 | (15.9) | (62.2) | 10.4 | 88.5 | 40 Min. after ingestion. |
| | Normal | 11:27-11:33 | 351.4 | 4.04 | 5.42 | 0.745 | 1.069 | 0.716 | (2.3) | (57.5) | 28.3 | 88.1 | 92 Min. after ingestion. |
| 3-15 | Clyde H. | 10:09-10:17 | 353.3 | 3.17 | 4.06 | 0.781 | .639 | 0.772 | 11.3 | 39.0 | 16.9 | 67.3 | Basal. 100% of average normal. |
| | 1.73 Sq. M. | 10:26 | | | | | | | | | | | Ingested 50 Gm. Glucose. |
| | 24 | 11:17-11:23 | 383.8 | 3.19 | 3.64 | 0.876 | .313 | 0.887 | 36.6 | 22.9 | 8.3 | 67.8 | 51 Min. after ingestion. |
| | Normal | 12:04-12:12 | 470.8 | 2.99 | 2.85 | 1.05 | .463 | 1.11 | 53.9 | 0.0 | 12.3 | 66.2 | 98 Min. after ingestion. |

4. The effect of the ingestion of calcium glycerophosphate upon the respiratory exchange is variable. In three diabetic subjects of the first type the respiratory quotients rose more readily than when glycerol was taken alone. It is impossible to interpret these results in terms of the oxidation of glycerol.

### Conclusions

The differences in the effects of glucose ingestion upon the respiratory exchange of patients with severe diabetes mellitus support the belief that there are different types of the disease corresponding to different mechanisms at fault.

In subjects with impaired ability to oxidize glucose hexose phosphate entered more readily into oxidation than did glucose alone.

# ON THE EXISTENCE OF MORE THAN FOUR ISOAGGLUTININ GROUPS IN HUMAN BLOOD

## Part II.*

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OUTLINE

Part I



* Presented before the Medical Society of the Johns Hopkins Hospital, October 16, 1922.

Note.—Contrary to the statement in Part I, our report has been divided into three instead of two portions, to facilitate publication. Like most serial stories, the individual installments taken separately are so incomplete as to be largely devoid of point or meaning; together and in the proper order they form a more or less connected narrative in which the plot is developed as logically as the facts and our inherent limitations permit. We venture to diverge from the accepted procedure in providing no synopsis of the matter presented in Part I.

## IV. Variations Within the Other Accepted Groups.

### 1.—*Group III.*

We have shown that for the one particular Group III blood (L.K.) used in our absorption tests (Section III, 3), the formula definitely appeared to be *BC—a* rather than the accepted one, *B—a*. Another Group III blood (A.H.) used in other absorption experiments behaved in the same way (*BC—a*).

There are two means of testing for the presence of agglutinin *C*. The more direct method would be by the agglutination of cells containing only agglutinogen *c*, but this was not feasible, as we have so far encountered no blood containing this one agglutinogen and no other. The other method is by the agglutination of cells containing agglutinogen *c*, in addition to some other agglutinogen, by serum from which agglutinins *A* and *B*, if originally present, have been absorbed. This method was open to us and was the one we used. It is tedious, however, and we have been unable to test more than a very few members of Group III, owing to other demands on our time. We believe that bloods having a structure different from *BC—a* are also to be found in Group III, and shall present evidence of this in Section VII.

The blood of our patient, C. T., might be considered a variant of Group III, with the formula *C—a*. Of the three members of her family (Gracie C., Alexander T. and G. V. C.), who were shown to react in the same way as C. T. in cross-agglutination tests with members of the four groups, not all have quite the same blood formula.

The niece, G.V.C., reacts in the same way as C.T., in that her serum agglutinates the cells of D.J. and of two other persons with blood like his; so we feel justified in assuming for her blood a formula like that of C.T. (*C—a*). As the two others do not agglutinate the cells of D.J., their bloods are apparently lacking in agglutinin *C* and their structure might be expressed as *O—a*.

2.—*Group IV.*

We have also been able to show that different sorts of blood are included in what is known as Group IV. This group is characterized by the inagglutinability of its cells and by the agglutinative power of its serum for the cells of the other three groups. As has been pointed out, absorption experiments indicated that the blood of one member of this Group (J.D.) was represented by the formula *ABC—o* (see Sect. III, 3). Another typical representative of Group IV (G.G.) reacted to the same tests in the same way. The blood of a third member of this group (S.P.) behaved quite differently. Preliminary cross-agglutination tests gave results shown in Table XXI.

TABLE XXI.

Cross-Agglutination Tests

| Serum | RED CELLS | | | | |
|---|---|---|---|---|---|
| | Gr. I (G.S.) | Gr. II (H.) | Gr. II (D.J.) | Gr. III (L.K.) | Gr. IV (S.P) |
| Gr. I (G.S.) | 0 | 0 | 0 | 0 | 0 |
| Gr. II (H.) | + | 0 | 0 | + | 0 |
| Gr. II (D.J.) | + | 0 | 0 | + | 0 |
| Gr. III (L.K.) | + | + | + | 0 | 0 |
| Gr. IV (S.P.) | + | + | + | + | 0 |

From Table XXI it is seen that all of these bloods behaved in a characteristic manner and each seemed representative of its group. The next step was to absorb Group IV (S.P.) serum with r.b.c from each of the other persons, and the absorbed serum was then tested against fresh suspensions of washed red cells. The results are shown in Table XXII.

TABLE XXII.

Results of Absorption Experiments

| Exp. No. | Serum | Absorbed with R.B.C. | Subsequent Agglutination of Red Cells | | | |
|---|---|---|---|---|---|---|
| | | | Gr. I (G.S.) | Gr. II (H.) | Gr. II (D.J.) | Gr. III (L.K.) |
| 1 | Gr. IV (S.P.) | Gr. I (G.S.) | 0 | 0 | 0 | 0 |
| 2 | Gr. IV (S.P.) | Gr. II (H.) | + | 0 | 0 | + |
| 3 | Gr. IV (S.P.) | Gr. II (D.J.) | + | 0 | 0 | + |
| 4 | Gr. IV (S.P.) | Gr. III (L.K.) | + | + | + | 0 |

From Table XXII it is seen that:

(a) In none of the experiments did agglutination occur with cells of the same type as those used for absorption; that is to say, the absorption was complete in each instance.

(b) After absorption with Group I (G.S.) cells, no agglutination occurred with any other cells in the series.

(c) Absorption with the cells of either Group II (H.) or Group II (D.J.) removed agglutinin for the other also, but left behind agglutinin for the cells of Group I (G.S.) and Group III (L.K.).

(d) Absorption with the cells of Group III (L.K.) removed agglutinin for his cells but left behind that for all others in the series.

These results are quite different from those presented in Table XVIII (Exp. 11 to 14). The serum of Group IV (S.P.), unlike that of Group IV (J.D.), was unable to distinguish between the red cells of Group II (H.) and those of Group II (D.J.), and, after absorption with the cells of Group I (G.S.), was entirely devoid of agglutinin for any of the cells used. Evidently the third agglutinin is lacking in her serum. Since we have previously evolved the formulæ for three of these five persons (Section III, 3), those appropriate for the others are easily derived.

| | | |
|---|---|---|
| Group I | (G.S.) | *O—ab* |
| Group II | (H.) | *A—b* |
| Group II | (D.J.) | *A—bc* |
| Group III | (L.K.) | *BC—a* |
| Group IV | (S.P.) | *AB—o* |

Thus it is seen that at least two different combinations exist which would ordinarily be classified under Group IV (*AB—o* and *ABC—o*). That still other combinations are included within this group will be pointed out in Sect. VII.

3. *Group I.*

In this group we have not as yet found any indication of a structure other than the traditional one (*O—ab*). We are inclined to think that this has been due largely to the fact that members of Group I are rare. In this work we have encountered but seven members of the group; of these only two were available for intensive study; and absorption tests indicated that the structure of these two bloods was identical. From our experience with Groups II, III, and IV, each of which has been found to include more than one type of blood, it is but reasonable to surmise that further study may reveal various combinations within this group as well.

## V. Relation of Autoagglutination to These Findings.

Very rarely one may encounter a blood whose cells are agglutinated by its own serum. This peculiar reaction—autoagglutination—occurs only at low temperatures; it disappears when the blood is warmed to 37° C., but reappears if the blood is cooled sufficiently. Such a blood was studied by Clough and Richter,[14] whose account is the most complete we have seen. They were able to separate the autoagglutinin from the isoagglutinin present in the serum of their patient and to show that, whereas the isoagglutinin was capable of agglutinating the red cells of certain other groups, the autoagglutinin was able to agglutinate the cells of any group, its own included. They

found that the autoagglutinin in the serum varied markedly in concentration from time to time, being active in relatively high dilution at one examination (1:500), but at subsequent tests, one month and two months later, causing a reaction only at a much lower level (1:16). As they also observed autoagglutination (less than 1:16) in the blood of a daughter of their patient, they suggested that it might be a hereditary characteristic.

In our studies we have found three bloods showing autoagglutination, all of them in one family,—the patient C.T., her brother Alexander T., and her sister Gracie C. In none of them was there high autoagglutinative strength at the time of any of our examinations. Even as compared with the titre of isoagglutinins in other bloods, it was distinctly feeble, since in a dilution of 1:2 it usually gave only a moderate agglutination either by the macroscopic or microscopic method. At some examinations it was more evident than at others, and in the blood of C.T., which was the one on which we made most observations, it could not be demonstrated at all on some occasions. We found that a short exposure to a lower temperature might give more striking results than were obtained in a much longer time at a somewhat higher temperature. Thus after 20 to 30 minutes in an ice-bath at 0° C., there was a more definite agglutination than occurred after several hours in the refrigerator (11 to 17° C.).

Our attention was first attracted to the autoagglutinin in the blood of C.T., by the difficulties encountered in washing her cells. When her whole blood was collected in citrate solution and placed for a time in the ice-box, the subsequent washing was complicated by the aggregation of the red cells into masses, sometimes of large size. As the temperature was raised, the masses could be disintegrated by gentle manipulation and, after the cells were completely freed from serum by repeated washings, no further clumping occurred. The autoagglutinin was active, not only against her own cells, but also against the cells of all other bloods tested, including members of the four accepted groups,—but not equally so. It was least active against the cells of Group IV, but was quite as active against her own cells as against those of any other blood.

Finding autoagglutination in the bloods of these three persons made us watch carefully for it in other bloods, but no additional instances were discovered. It also made us scrutinize our experimental procedures very closely to avoid the possibility of mistaking autoagglutination for isoagglutination.

In this connection the following points seem worthy of mention.

(1) Of the three persons in whom autoagglutination was demonstrated, two (Gracie C., and Alexander T.) are not credited with any positive reactions due to isoagglutination. The third (C.T.) is described as exhibiting isoagglutination with only one type of cells. Under conditions suitable for the manifestation of autoagglutination, the serum of C.T. clumped any sort of human cells, her own quite as well as those of another; but at higher temperatures it agglutinated only the cells of D.J. and other so-called members of Group II with blood like his. The autoagglutinin was sometimes absent; the isoagglutinin for the cells of D.J. was present at each examination.

(2) All of the bloods studied were examined by extensive series of cross-agglutination tests, and in every series each serum was tested against each set of cells. Under such circumstances the occurrence of autoagglutination is sure to attract attention.

(3) After the primary reading, several additional readings were made on all our tests after subjection to various temperatures, a procedure calculated to discriminate between reactions due to the two types of agglutinins.

For these reasons we believe that the occurrence of autoagglutination has not been a complicating factor in our observations concerning the existence of bloods giving unusual isoagglutinin reactions.

### VI. Classification of Blood Groups.

In Section II, 6, we have pointed out that the theory of Landsteiner, accepted and modified by others, was quite adequate to explain the behavior of four blood groups, as dependent upon the action of two agglutinins and two agglutinogens. It is true that Hektoen [7] and Moss [8] assumed the presence of three agglutinins, but several writers have pointed out that such an assumption was unnecessary to explain the reactions observed. The same criticism is not valid when applied to the results of the work reported here; the more complicated relationships which we have shown to occur in the isoagglutinin groups are not susceptible of explanation on the earlier and simpler basis.* Our observations demand the assumption of a third isoagglutinin and a corresponding isoagglutinogen in the bloods of some persons ordinarily classified in one or other of the four accepted groups.

We have called attention to the fact that the four formulæ used to indicate the structure of the accepted groups by no means exhausted the possible combinations of two agglutinins and two agglutinogens (Section II, 6). With the addition of a third agglutinin and a third agglutinogen the number of possible combinations is greatly increased (see Table XXIII).

* The work of Koeckert,[15] for instance, offers convincing evidence that the theory of 3 agglutinins and 3 agglutinogens is untenable in the form in which it was evolved by Moss to explain the behavior of bloods in the four accepted groups. With the experiments of Koeckert, so far as they go, our observations are in complete accord (see Tables X and XVIII). His experiments, however, do not controvert the suggestion which we have advanced as an explanation of the reactions of certain unusual bloods.

TABLE XXIII.

Combinations of 3 Agglutinins and 3 Agglutinogens; Mathematical Possibilities.

| Comb. No. | Contains | | Comb. No. | Contains | | Comb. No. | Contains | |
|---|---|---|---|---|---|---|---|---|
| | Agglutinin | Agglutinogen | | Agglutinin | Agglutinogen | | Agglutinin | Agglutinogen |
| 1 | *O* | *a* | 23 | *BC* | *c* | 45 | *AB* | *bc* |
| 2 | *A* | *a* | 24 | *ABC* | *c* | 46 | *AC* | *bc* |
| 3 | *B* | *a* | 25 | *O* | *ab* | 47 | *BC* | *bc* |
| 4 | *C* | *a* | 26 | *A* | *ab* | 48 | *ABC* | *bc* |
| 5 | *AB* | *a* | 27 | *B* | *ab* | 49 | *O* | *abc* |
| 6 | *AC* | *a* | 28 | *C* | *ab* | 50 | *A* | *abc* |
| 7 | *BC* | *a* | 29 | *AB* | *ab* | 51 | *B* | *abc* |
| 8 | *ABC* | *a* | 30 | *AC* | *ab* | 52 | *C* | *abc* |
| 9 | *O* | *b* | 31 | *BC* | *ab* | 53 | *AB* | *abc* |
| 10 | *A* | *b* | 32 | *ABC* | *ab* | 54 | *AC* | *abc* |
| 11 | *B* | *b* | 33 | *O* | *ac* | 55 | *BC* | *abc* |
| 12 | *C* | *b* | 34 | *A* | *ac* | 56 | *ABC* | *abc* |
| 13 | *AB* | *b* | 35 | *B* | *ac* | 57 | *O* | *o* |
| 14 | *AC* | *b* | 36 | *C* | *ac* | 58 | *A* | *o* |
| 15 | *BC* | *b* | 37 | *AB* | *ac* | 59 | *B* | *o* |
| 16 | *ABC* | *b* | 38 | *AC* | *ac* | 60 | *C* | *o* |
| 17 | *O* | *c* | 39 | *BC* | *ac* | 61 | *AB* | *o* |
| 18 | *A* | *c* | 40 | *ABC* | *ac* | 62 | *AC* | *o* |
| 19 | *B* | *c* | 41 | *O* | *bc* | 63 | *BC* | *o* |
| 20 | *C* | *c* | 42 | *A* | *bc* | 64 | *ABC* | *o* |
| 21 | *AB* | *c* | 43 | *B* | *bc* | | | |
| 22 | *AC* | *c* | 44 | *C* | *bc* | | | |

If we eliminate all combinations which would indicate the presence of an agglutinin and its corresponding agglutinogen in the same blood, there remain 27 which we may for the present regard as biological possibilities (see Table XXIV).

TABLE XXIV.

Combinations of 3 Agglutinins and 3 Agglutinogens; Biological Possibilities.

| Comb. No. | Contains | | Comb. No. | Contains | | Comb. No. | Contains | |
|---|---|---|---|---|---|---|---|---|
| | Agglutinin | Agglutinogen | | Agglutinin | Agglutinogen | | Agglutinin | Agglutinogen |
| 1 | *O* | *a* | 10 | *A* | *c* | 19 | *O* | *abc* |
| 2 | *B* | *a* | 11 | *B* | *c* | 20 | *O* | *o* |
| 3 | *C* | *a* | 12 | *AB* | *c* | 21 | *A* | *o* |
| 4 | *BC* | *a* | 13 | *O* | *ab* | 22 | *B* | *o* |
| 5 | *O* | *b* | 14 | *C* | *ab* | 23 | *C* | *o* |
| 6 | *A* | *b* | 15 | *O* | *ac* | 24 | *AB* | *o* |
| 7 | *C* | *b* | 16 | *B* | *ac* | 25 | *AC* | *o* |
| 8 | *AC* | *b* | 17 | *O* | *bc* | 26 | *BC* | *o* |
| 9 | *O* | *c* | 18 | *A* | *bc* | 27 | *ABC* | *o* |

Of these 27 theoretically possible combinations, our work has indicated the actual existence of 8, as follows:

1. *O—a* (Gracie C. and Alexander T.)
3. *C—a* (C.T. and G.V.C.)
4. *BC—a* (L.K. and A.H.)
6. *A—b* (J.H. and many others)
13. *O—ab* (Y.O. and G.S.)
18. *A—bc* (D.J. and 15 others)
24. *AB—o* (S.P.)
27. *ABC—o* (J.D. and G.G.)

This leaves 19 of the 27 possibilities without a representative. That some at least of these 19 combinations probably exist, we may be able to show in the following section of this report.

## VII. Indications From the Observations of Other Workers of the Existence of More Than Four Isoagglutinin Groups.

In view of our findings, it may seem surprising that the validity of the four accepted groups has not been seriously questioned during the past ten or twelve years, during which blood matching and grouping has been carried on to such an enormous extent. It should be remembered, however, that not only are the tests in common use based upon the assumption that all human beings above the period of infancy belong to some one of the four known groups, but these tests are so devised as to cause every blood tested to fall into one or other of these groups. Most persons engaged in performing isoagglutination tests do so merely to determine compatibility of bloods and availability of donors for transfusion. Those who actually do grouping use one, and usually only one, of the accepted short-cut methods. Under these conditions, while discrepancies might be encountered, it is extremely unlikely that an unusual group would be recognized. The chances of making such an observation would be greatly increased in the case of those performing extensive series of cross-agglutination tests in which bloods from all of the four groups were used; even then a very long series might be required before a blood with exceptional reactions was discovered. None such was observed by Moss in performing 5 successive series of cross-agglutination tests, each series containing blood from 20 different persons. That such exceptional bloods may be encountered and still be overlooked is apparent from a careful study of Janský's tables.

1.—*Janský.*

In his work Janský [6] used sera from 30 psychiatric patients; two of these patients were epileptics and from each of them he secured an additional specimen of serum just after an attack, thus having 32 specimens of serum from 30 persons. The 32 sera were diluted 1 to 5 and used to test the whole blood of 99 persons including the 30 who furnished the sera. He made his preparations on slides which were then examined macroscopically. Each blood was tested with each of the 32 sera, except in 8 instances, making a total of 3160 tests, all of which are tabulated in his article. On the basis of the results obtained, he classified his 32 sera as follows: Group I (Moss Gr. IV),—12; Group II,—12 (including two duplicates); Group III,—6; and Group IV (Moss Gr. I),—2. It is easy for the reader to analyze his tables and assign similar groupings to most of the other persons whose serum was not used, but whose whole blood was tested with the

32 specimens of serum. The blood of some of these persons, however, did not react in such a manner as properly to permit their inclusion in any of the four groups; of these exceptional bloods—to which he made no reference in his text—there are two in particular to which we wish to call attention.

The whole blood of No. 7627-Š.J., a patient with melancholia, was tested with the 32 specimens of serum previously mentioned. The tabulated results show that red cells of this patient were agglutinated by each of the 12 Group I (Moss Gr. IV) sera used, and by these only. The results were negative with the other 20 sera representing Group II, Group III and Group IV (Moss Gr. I). This indicates that the red cells of No. 7627-Š.J. were devoid of agglutinogen capable of reacting either with agglutinin *A* present in Group II serum, or agglutinin *B* present in Group III serum. There was present in these red cells, however, an agglutinogen capable of reacting with an agglutinin present in Group I serum (Moss Gr. IV) but absent from the sera of Groups II and III. This necessitates the assumption of an agglutinogen other than *a* or *b* in the red cells of the patient No. 7627-Š.J., and of an agglutinin in addition to *A* and *B* in the serum from each of the 12 members of Group I (Moss Gr. IV). As the serum of No. 7627-Š.J. was not used in any of Janský's tests, we can form no surmise concerning its agglutinin content other than that it might have been that indicated by any one of the four combinations numbered 9 to 12 in Table XXIV (*O—c*; *A—c*; *B—c*; *AB—c*); it is sufficient for our purpose to represent this unusual blood by the formula *?—c*. Whatever its agglutinin content, it is obvious (1) that this blood was different from that of the members of Janský's four groups; (2) that it forms an exception sufficiently definite to prove the existence of an additional group; and (3) that more than two isoagglutinins and two isoagglutinogens are present in human blood.

It has been mentioned that the cells of patient No. 7627-Š.J. were not agglutinated by any sera except those of Group I (Moss Gr. IV) and we have presented our reasons for assuming the presence of agglutinogen *c* in these red cells and of agglutinin *C* in the sera which agglutinated them. From these premises it is logical to conclude that agglutinin *C* was absent from the serum of the 6 members of Group III, all of which failed to agglutinate the cells of No. 7627-Š.J. The formula for these members of Group III, therefore, would be that represented by combination 2 in Table XXIV (*B—a*), rather than that which we found in the two Group III bloods which we studied by means of absorption tests (Sect. III, 3; Sect. IV, 1).

Other discrepancies are to be found in his tables, as for instance, in the behavior of the blood patient No. 7524-F.B. (progressive paralysis). This blood was classified as belonging to Group III (page 116) but the tables show that the red cells were not agglutinated by any serum, in other words they reacted like the cells of Group I (Moss Gr. IV). The serum of this patient agglutinated the cells of Group II and Group IV (Moss Gr. I), but it agglutinated the cells of only 6 of the 19 bloods in the series whose cells behaved otherwise as Group III, and failed to agglutinate those of the other 13. Apparently the blood of No. 7524-F.B. in its reactions resembled Group I (Moss Gr. IV) more closely than the group to which Janský assigned it, but differed from Group I (Moss Gr. IV) in that it was deficient in agglutinating power for some Group III cells.

This in turn calls attention to the fact that the 19 bloods whose cells reacted like those of Group III were not all of the same type. The agglutinogen content of the 6 that were agglutinated by serum of No. 7524-F.B. must have been different from that of the 13 that were not so agglutinated, yet to every other serum except this one, the 19 bloods reacted consistently. This is in many ways like our own experience already dwelt upon at some length; had we not had at our disposal one exceptional blood (C.T.), we would never have suspected that there was anything unusual about that of D.J.

Although these rather obvious exceptions to his general rules apparently escaped the attention of Janský, indications are not lacking that others have encountered somewhat similar discrepancies.

2.—*Ottenberg.*

In 1911 this author[13] describing the characteristics of Group III wrote as follows: "Members of this group sometimes show slight individual irregularities, the cells now and then failing to be agglutinated by the sera of some members of the second group, although being agglutinated by others, and the sera occasionally agglutinating the cells of some but not all other members of the third group itself."

Such observations, if correct, would certainly suggest that arbitrary classification of bloods so unlike in the same group is both inexact and illogical.

From an earlier report with Epstein,[16] in which they classified bloods into 3 groups we read, "This grouping was remarkably regular. Out of all the tests there was really not one which did not fit into it. There was one exception in which with repeated trials the red blood cells of group 2 were not agglutinated by the serum of a blood which clearly belonged to group 1 since it was not agglutinated by any other blood." The reactions of this exceptional blood (No. 45) are shown in their table on page 121.

What are we to think of a so-called Group IV blood, for this is the equivalent of their "group 1," which agglutinates Group III cells and fails to agglutinate those of the four members of Group II against which it was tested? It might be added that it also agglutinated the cells of Group I, as there was in the series an unrecognized member of this group (No. 49) and probably another (No. 48).

Taken at their face value, the results indicate an absence of agglutinogens *a* and *b* in the red cells of this exceptional blood, since they are not agglutinated by the sera of Groups II and III, and also an absence of agglutinin *B* in the serum, since it does not agglutinate the cells of Group II. As to the possible structure of such a blood we can only suggest that it might be that of combination 10, 21 or 25 shown in Table XXIV (*A*—*c*; *A*—*o*; *AC*—*o*).

Attention might also be called to the behavior of No. 47 classified as "group 3" which agglutinates the cells of the 4 members of "group 2" in the series and those of one unrecognized member of Group I (No. 49), but fails to agglutinate those of what is almost certainly another member of Group I, since its serum is apparently devoid of agglutinative power (No. 48). This also, if taken at its face value, would indicate that No. 48 and No. 49, although alike in the absence of agglutinins from their sera, differed in the agglutinogen content of their cells. Since the cells of both were agglutinated by group 1 (Group IV) and group 2, they apparently possessed at least one agglutinogen in common (*a* or *ac*) ; since one was agglutinated by the serum of the only member of Group III against which it was tested and the other was not, there was evidently an agglutinogen in the cells of one which was lacking in the other (*b*). The structure of these bloods might be indicated as possibly being *O*—*ab* or *O*—*abc* for No. 49 and *O*—*a* or *O*—*ac* for No. 48 (Combinations 13, 19, 1 and 15 in Table XXIV). Two of these are combinations which we have found in our own work, *O*—*ab* and *O*—*a* (see Sect. VI).

3.—*Brem.*

In a report in 1916 Brem [11] wrote, "Another precaution that must be observed is that the known Group 2 or Group 3 blood which is used for determining the group of another blood must contain in the serum strong agglutinins. The strength or quantity varies in different serums even of the same group, and we were led into serious error on another occasion by using a known Group 2 blood, which was weak in agglutinins, for determining the group of a donor. The known Group 2 corpuscles were not agglutinated by the donor's serum and the known Group 2 serum apparently did not affect the donor's corpuscles, that is the donor apparently belonged to Group 2, which was the group desired for transfusion. The reaction which resulted in this instance, also, was most alarming. Afterward we regrouped the donor's blood with another Group 2 blood and found that the known Group 2 serum agglutinated the unknown corpuscles, that is, the donor's blood belonged to Group 1 and was unsuitable for the transfusion."

Here was a "known Group 2 blood" with serum which failed to agglutinate the cells of "Group 1." It would be very helpful to know (1) whether this serum was fresh, or old and deteriorated, and (2) whether the serum from this person was ever able to agglutinate these particular "Group 1" cells, before or after the date of this failure, but information on these points is lacking. If this was a transient deficiency in agglutinative power, or one dependent upon deterioration of stock serum, it does not concern us in this connection.

On the other hand, if fresh serum from this "known Group 2" was never able to agglutinate the cells of this "Group 1" blood, in other words, if the weakness of agglutinating power of the serum amounted to continued and consistent inability to agglutinate cells readily agglutinated by other "known Group 2" sera, it suggests a blood devoid of agglutinin *A* but containing agglutinogen *b*. Such a blood (*O*—*b*) would correspond to combination 5 in Table XXIV. Such a blood would naturally be classified as belonging to Group II by the method of cross-agglutination with the cells and serum of a veritable member of Group II. Cross-agglutination with the cells and serum of Group III would assign it to Group I (see Table IV). In reality it properly belongs in neither group.

4.—*Meleney, Stearns, Fortuine and Ferry.*

In their report on "Post-transfusion Reactions" these writers say [17] "Specimens of blood belonging to Group III are occasionally met with, Miss Olmstead tells us, whose serum has no agglutinins for Group II. The final test for these specimens to distinguish them from Group IV" [Moss Gr. I] "which also has no agglutinins for Group II, must be their ability to agglutinate the cells of Group IV" [Moss Gr. I] "and the inability of the serum of Group III to agglutinate their cells. As Group IV" [Moss Gr. I] "is usually not available, the latter point must usually be the deciding one." A blood with the formula *A*—*o* would fulfill these conditions, namely, (1) inability to agglutinate the cells of Group II, (2) ability to agglutinate the cells of Group IV (Moss Gr. I), and (3) insusceptibility of red cells to agglutination by the serum of Group III; but as the serum would be able to agglutinate the red cells of Group III, it is difficult to see how such a blood could be assigned to Group III if this test were carried out.

5.—*Happ.*

The work of Happ[18] and others, who preceded him in the same line of investigation, has furnished much information concerning the development of isoagglutinin groups in the blood of infants and children. From Happ's conclusions the following are of interest to us at present:

"2. The grouping as present in adults is rarely present in blood from the umbilical cord."

"3. At birth and during the first month of life isoagglutination is rarely present, but the percentage of infants in whom the isoagglutinin group is established increases with age, so that after 1 year the group is usually established, and after 2 years is always present as in adults."

"4. The grouping is established in the corpuscles before it is established in the serum: *i.e.* the corpuscles acquire agglutinophilic receptors before the serum acquires agglu-

tinin. Therefore, Group I is the first group to be formed and Group IV is the last."

"5. The early grouping in the corpuscles before the group is established in the serum is liable to change by the acquisition of new receptors."

"6. When the grouping has been established in both serum and corpuscles it does not change."

Conclusion No. 5 suggests an interesting possibility. In the successive development of agglutinogens,—the appearance of one often preceding the development of another by a considerable interval—the red cells of an infant might at one stage contain agglutinogen *c* alone, before its presence was obscured or overshadowed by the development of agglutinogen *a* or *b*.

His first table (p. 319-320) shows the results of tests performed with 49 different bloods from the umbilical cord or from infants up to one month old.. The sera and cells were tested against the cells and sera of adults representing each of the four accepted groups. Three of these 49 bloods showed a very interesting reaction;—at one stage the red cells from each were "definitely" agglutinated by the serum of Group IV but not by the sera of Groups I, II or III. One of these bloods (No. 12; negro; female; tested June 6, 1914; aged 7 days) was again examined 10 days later, at which time the red cells were "definitely" agglutinated by serum III as well as serum IV, and so were assigned to Group II. Another (No. 15; white; male; tested June 8, 1914; blood from umbilical cord at birth) was also examined 10 days later, at which time the red cells were "definitely" agglutinated by serum II as well as by serum IV, and so were classified as Group III. The third blood giving this unusual reaction (No. 47; white; male; tested May 25, 1916) was tested only once, at a time when the infant was seven days old.

These findings may be interpreted in more than one way. Was the Group IV serum stronger than the others? Did it contain more agglutinin *A* than serum II, and more agglutinin *B* than serum III? A serum of high agglutinative power might cause a reaction with feebly agglutinable cells, not produced by weaker sera. This suggestion cannot be entirely dismissed but would have greater force if agglutination of these cells by any serum, when it occurred at all, had been reported as anything less than "definite." There is quite as much to recommend another theory which implies a qualitative rather than a merely quantitative difference, (1) in the cells of the same infant at different periods, and (2) in the different sera. If the latter explanation is correct, we might conclude from the behavior of these three bloods that:

(a). There was present in the red cells when first tested, an agglutinogen incapable of reacting with either agglutinin *A* or agglutinin *B*, but readily demonstrable by means of serum from Group IV,—a serum which as we have shown, not infrequently contains agglutinin *C*. This agglutinogen was, in all probability, *c*.

(b). Agglutinogen *c* was demonstrable in these red cells before the appearance of either agglutinogens *a* or *b*, and so, for a period, was the only one present.*

(c). The result of tests made after a lapse of 10 days, illustrates the way in which the presence of agglutinogen *c* may be obscured by the presence of agglutinogens *a* or *b*. If the earlier tests had been omitted, the presence of agglutinogen *c* would have escaped recognition.

6.—*Unger.*

In 1921 Unger [19] wrote as follows:

"Although, in the majority of cases, the blood of donor and patient belonging to the same group are compatible, nevertheless, the number of exceptions are sufficiently great to warrant a different procedure and greater caution. In the course of performing many thousands of tests preliminary to transfusion, I have occasionally noted that, although the donor and patient are of the same group, when the bloods are tested one against the other a small number of agglutinated clumps of blood cells will be seen."

"Each time this phenomenon was observed, the same samples of serum of Groups II and III were used to determine the group of the patient, and all donors who were tested against this case. Certain donors were found habitually to give this reaction. Although their cells fell definitely into Groups II or III, their serum gave minor agglutinative reactions against cells of patients of the same group. The microscopic method was used in every case; in none was there any rouleaux formation."

"Although ordinarily such donors are rejected, urgent need has forced me to transfuse patients in which this phenomenon occurred. In each case there was a sharp reaction—chill and fever—undoubtedly due to the occurrence of agglutination. One of these patients I subsequently transfused with a donor whose blood was perfectly compatible, as viewed by the microscope, and no reaction whatever occurred."

"The recognized group of an individual is established by the presence of a "chief" or "major" agglutinin in his serum. Agglutination of cells by serums of the same group is probably due to a "para" or "minor" agglutinin, which causes a lesser degree of agglutination. The transfusion of such blood gives rise to clinical reactions. This disagreeable result, however, can be eliminated by testing the blood of every prospective donor directly against the patient's blood and by careful microscopic examination of the mixtures."

"It is believed commonly that the cells of Group IV cannot be agglutinated by any serum. A person of this group is accepted as a 'universal donor.' By this term is meant

* That this particular stage may persist in the blood of older persons, is illustrated by the behavior of the red cells of Jansky's patient No. 7627-š. J., which were agglutinated only by the serum of Group IV (see Sect. VII, 1). It is true that the age of the patient was not stated but it is improbable that a young child would be diagnosed as suffering from "melancholie."

a donor who in an emergency may furnish blood to any patient regardless of group, on the theory that danger arises only when the donor's cells are agglutinated and that agglutination of the patient's cells may be disregarded. In nine patients, however, serum was found to agglutinate cells of certain Group IV donors. Six of these patients belonged to Group IV, one to Group II, and in two others unfortunately the type was not determined. A result of this kind demonstrates the inadequacy of 'typing' merely the patient, and the necessity of testing the blood of donor and patient one against the other. Five patients showed the phenomenon of auto-agglutination—that is, the cells of the patient were agglutinated by his own serum; the four others apparently contained 'minor' agglutinins for Group IV cells. . . . . For patients whose blood contains minor agglutinins for Group IV cells, search must be made until a proper donor is found."

This quotation serves to emphasize the futility of regarding as conclusive the classification of bloods by a method with such obvious limitations. It also emphasizes a point which we consider self-evident; *whatever their previous classification, bloods which cross-agglutinate do not belong to the same group.*

We shall omit detailed analysis of this interesting and suggestive communication, selecting merely the final paragraph of the quotation for further comment. In this paragraph we are told: "In nine patients, however, serum was found to agglutinate cells of certain Group IV donors. . . . . Five patients showed the phenomenon of auto-agglutination, . . . . . the four others apparently contained 'minor' agglutinins for Group IV cells." These bloods, particularly the "four others" just referred to, which were capable of agglutinating the cells of "certain Group IV donors," seem to have impressed the author as being unusual. It is an impression which we share; they were unusual; any blood capable of agglutinating Group IV cells is unusual. We desire to direct attention, however, to the "certain Group IV donors" whose cells were agglutinated. One of the criteria for the recognition of Group IV is that the cells of this group are inagglutinable by the serum of their own or any of the other three accepted groups, yet there are "certain Group IV donors" which form an exception to this rule. Since they so obviously differ from the common run of IV's, their inclusion in this group must rest upon sentiment and tradition only. How were they assigned to Group IV in the first place? The context leads us to believe that it was by the use of sera from Groups II and III; and to have been classified as Group IV by this means, there must have been no agglutination by either serum. This suggests that the red cells of these unusual bloods lacked agglutinogens *a* and *b,* and that their eventual clumping by the "minor agglutinin" in the serum of "the four others" was produced by reaction with agglutinogen *c*. Lacking information concerning the behavior of the sera of these unusual bloods we have no means of surmising their agglutinin content. Our conjecture regarding the formula which might express such characteristics as we are warranted in assuming, cannot go beyond that used in the case of Janský's patient No. 7627-š.j., *?—c*.

7.—*Schütze.*

In 13 Group II bloods, this author [20] found 5 whose cells were feebly agglutinated and 9 whose cells were strongly and equally agglutinated by the serum from a member of Group IV. Absorption of this Group IV serum with the cells of the first type (feebly agglutinated) removed much but not all of the agglutinin for these, and still left the agglutinative power for the other type (strongly agglutinated) practically intact. Absorption of the Group IV serum with the strongly agglutinated cells removed most of the agglutinin for these and all of that for the feebly agglutinated cells. It is to be noted that in neither experiment was the absorption complete for the type of cells used for absorption. He attributes his results solely to quantitative differences in agglutinability of the two types of Group II cells, but it is evident that they might just as readily have been due to qualitative differences. His results are rather reminiscent of those which we obtained with the cells of Group II (H.) and Group II (D.J.) (see Sect. III, 3). It might be urged that his explanation might do equally well for our observations if we had not controlled them in another way. The serum of the patient C.T. never agglutinated the cells of Group II (H.) and others of this type; it always agglutinated cells of the type of Group II (D.J.) and of this type only. This is strong evidence of a qualitative rather than a merely quantitative difference in the two types of Group II cells.

8.—*Hooker and Anderson.*

In the admirable paper in which Hooker and Anderson [21] describe the production of group-specific heteroagglutinins by the immunization of rabbits with human blood from each of the four groups, they admit surprise at securing a serum of even greater group-specificity with the cells of Group IV—which are supposed to contain no isoagglutinogen—than with those of the other three groups.

Certain antigenic properties were assumed to be common to red cells of the four groups. Since the immunized rabbit sera were to a greater or lesser extent group-specific, it was apparently likewise assumed that the isoagglutinogens characteristic of the red cells of Groups I, II and III were capable of acting also as heteroagglutinogens, and the absorption experiments add weight to this assumption. This the authors could understand, but were unable to account for the fact that their Group IV cells evidently also contained a group-specific agglutinogen. Inasmuch as they considered the question of the existence of "subgroups" as still an open one, they took steps to reduce "the number of variables"—presumably those occurring in members of the same group—by using the

blood from only one person in each group for the immunization of their rabbits. We can merely speculate as to the formula of this blood used as a representative of Group IV. In its reaction with the bloods of the other groups it behaved in a characteristic manner; if its formula had been *AB—c,* it would probably have behaved no less characteristically and the results obtained on immunization would be more readily understood. The following quotation may be readily reconciled with our suggestion. "That a specific isoagglutinogenic element is possessed by many members of Group I" [Moss Gr. IV] "is suggested by the observation that after transfusion of Group I blood" [Moss Gr. IV] "a considerable search is often necessary before inagglutinable bloods can be found for subsequent transfusions."*

We have neither desired nor attempted to present every suggestion to be found in the literature concerning the existence of additional isoagglutinin groups, but have contented ourselves with a few examples illustrating various sorts of exceptions to the accepted rule. From these citations it is evident that other workers have encountered bloods which did not behave in the traditional way. Concerning some of these exceptional bloods there is sufficient information available to form a reasonable idea as to their agglutinin or agglutinogen content. The formulæ thus evolved have been shown to correspond with certain of the combinations considered as biological possibilities and presented in Table XXIV. We do not wish to lay too much stress on the conjectures advanced to explain the behavior of these bloods, but it is difficult to escape the conclusion that other combinations exist beside the eight which we have encountered in our own work. (Sect. VI.)

We might quote instance after instance from the published and unpublished experience of others in which the bloods of persons, shown to belong to the same group by the ordinary tests, exhibited isoagglutination when matched against each other. All of which serves merely to accentuate the fact that by the methods in general use, every blood is bound to fall into one of the four accepted groups; but within some, perhaps within each of these four groups, are included bloods of diverse sorts, having sufficient in common to secure assignment to the same group but enough dissimilarity to manifest incompatibility, oftentimes on cross-matching or on transfusion, but which may escape recognition even then.

(To be concluded.)

* The work of Langer[22] we have not overlooked, but intentionally omitted, as his results are too bizarre for acceptance, even by us. In cross-agglutination tests with bloods from 10 persons, he found 9 different reaction-combinations; in another similar group of 10 he found 8 different combinations. We are asked to believe, in effect, that 17 blood groups were found in the examination of only 20 persons, apparently selected at random. These results have not been confirmed by any subsequent observer. Langer maintained also that he had demonstrated the presence of not less than 6 different isoagglutinins in one serum. We have felt, therefore, that citations from his work would not add to the credibility of our own, and have preferred to confine our references to the work of authors whose observations inspired more confidence.

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# STUDIES ON THE INFLUENCE OF PREGNANCY IN SYPHILIS

## I. THE COURSE OF SYPHILITIC INFECTION IN PREGNANT WOMEN *

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Colles' law,[1] formulated in 1837, represents the first recognition of the fact that in addition to the pernicious effects of syphilis on the product of conception, there is also a modifying influence exercised by pregnancy on the course of syphilitic infection. This law, which states that a non-syphilitic woman may bear a syphilitic child and that, since she may nurse her own child with impunity though others may be infected by it, she is therefore immune from infection, implies that the fetus is infected directly by the father. The validity of Colles' law and of the paternal transmission of syphilis is still unsettled. The purpose of this paper is to show from a correlation of the clinical and experimental evidence in the literature that in all probability neither of these hypotheses is valid. If this be admitted, it will then be shown by a clinical study of pregnant syphilitic women that pregnancy may cause alterations from the typical response to infection with syphilis, such as complete suppression of all early lesions or a disturbance of the usual time relations of the primary, secondary, and tertiary periods of the disease. Furthermore, it will be demonstrated that the protection from the manifestations of syphilis afforded by pregnancy often extends over a long period of time. It will be shown also that the Wassermann test in the blood during the pregnant state behaves in a manner different from that in normal women.

In the older literature, it has been generally considered that paternal transmission of syphilis to the fetus was not only possible but frequent, the main points of the argument being the obvious freedom of mothers from signs of infection and the fact that anti-syphilitic treatment of the father alone sometimes resulted in a healthy infant.[2] As corroborative evidence of this possibility, it may be pointed out that several investigators have demonstrated the infectivity for animals of seminal fluid from syphilitic men, though only a small percentage of attempts have been successful,[3] and these usually from patients with florid secondary syphilis. Successful inoculations with the semen of syphilitics in the tertiary or in the latent stage have been very few indeed.[4] Recently, treponemes have been microscopically demonstrated in semen.[5] The great frequency of testicular involvement in late syphilis has also been emphasized.[6]

However, attempts to inoculate the symptomless mothers of syphilitic children with infectious material from patients with primary or secondary syphilis were unsuccessful (Caspary,[7] Neumann,[8] Finger.[9]) By means of animal inoculation, Buschke [10] succeeded in demonstrating *Treponema pallidum* in the inguinal glands, and Uhlenhuth and Mulzer [11] found it in the milk, of such mothers. Moreover, with the introduction of the Wassermann reaction, it developed that about 90 per cent of the mothers of syphilitic children presented positive tests in the blood.[12]

It was further pointed out that paternal infection of the ovum was highly unlikely. Bab [13] and Trinchese [14] showed that only four possibilities of infection of the fetus exist: (1) the spermatozoon may carry the treponeme into the ovum; (2) the treponeme may be contained in the semen and, though not in intimate contact with the sperm cell, may penetrate the ovum simultaneously with it; (3) the ovum may contain treponemes prior to fertilization; or (4) the fetus may be infected through the maternal circulation. The first possibility is practically inconceivable because of the relative size of treponeme and spermatozoon (unless one presupposes a much smaller form of organism as yet undiscovered), the almost certain early death of an ovum infected in this way, and the fact that, if the treponeme could thus invade such a minute part of the maternal organisms as the ovum, it is impossible that the mother herself could escape infection. The second requires a constant synchronization of penetrating power between the sperm cell and the treponeme, which is equally fanciful. As for the third, even if we leave out of consideration the probable fate of such an infected ovum, treponemes have been found in ova too infrequently to convert this possibility into a probability. Trinchese further points out that in abortions before the fifth month evidences of syphilitic infection of the fetus are always lacking. Inasmuch as later fetal syphilis consists of an overwhelming spirochetal sepsis, he concludes that infection of the fetus must occur in the latter half of pregnancy, and that it must enter from the maternal circulation. That this mode of infection of the fetus is possible is shown by the animal experiments of Uhlenhuth and Mulzer.[15] They demonstrated that, in pregnant rabbits, the placenta is easily traversed by virulent treponemes inoculated intravenously, and that the organisms may be

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readily recovered from the fetal organs or the fetal portion of the placenta.

The refractory state of the mothers of syphilitic children to re- or super-inoculation, the demonstration that they may be carriers of treponemes in lymphoid tissues (analogous to the findings in animals and humans definitely known to be latent syphilitics),[16] the almost constant occurrence of a positive Wassermann reaction in their bloods, the evidence against the occurrence of infection of the young ovum, and the demonstration of the placental permeability to treponemes has finally settled the question of Colles' law for most workers. A few, however, remain still unconvinced,[17] and, from the tone of current literature, there are others who are at least doubtful.

Recent experimental evidence offered by Brown and Pearce[18] is here of the utmost importance, and deserves quotation. They say:—

"Normal rabbits, whether male or female, react to intradermal inoculation of the vulva or sheath by the prompt development of characteristic indurated lesions at the site of inoculation and a well marked lymphadenitis.

"Of eight pregnant females inoculated in the same way, only four of them showed any clinical signs of infection whatsoever; in three of these the reaction consisted of a very slight and transient infiltration at the site of inoculation, unaccompanied by lymphadenitis; one of these animals showed no constitutional disturbance, but the two in which the local reaction was most marked, showed profound constitutional disturbances as well. . . . . The only inoculation of a pregnant animal which gave rise to a lesions comparable to those of the controls was made during the middle of pregnancy, but the lesion did not appear until towards the end of lactation."*

The experiment just cited shows that a pregnant rabbit inoculated at the time of conception fails to react to infection in the same manner as normal controls. There may be created in the animal a condition quite analogous to that in the human who apparently fulfills the requirements of Colles' law,—a complete absence of signs of syphilitic infection, although, in the case of the rabbit, infection is definitely known to exist. These data provide the missing link in the chain of circumstantial evidence against the validity of Colles' law. To complete the case, it is necessary only to show that a large proportion of Colles' women are actual treponeme carriers, in spite of their own freedom from disease. In a subsequent paper of this series this point will be dealt with.

There have been reported a few cases of exceptions to Colles' law, the mother of a syphilitic child having subsequently acquired primary and secondary syphilis. As Carle[19] points out, however, these cases are wholly unconvincing to a trained observer and may probably be dismissed altogether. It is more difficult to explain why treatment of a syphilitic father will interrupt a series of syphilitic fetuses with a healthy child. The well known capriciousness of syphilis in attacking the fetus may be the explanation.

It can now be shown that in the human, as in the animal, definite alterations in the course of syphilis are caused by pregnancy. The most important consists in a complete suppression of the usual early lesions of the disease, provided infection and impregnation coincide, and this condition occurs when infection is known to be transmitted in the usual manner, by direct contact. The peculiarities of the disease in these women probably depend, therefore, on the factor of pregnancy, and not on the method of infection.

*The Frequency of Latent Syphilis in Women.*—That there are variations in the clinical types of syphilis in males and females has long been known. The Wassermann reaction has provided an additional fact,—the frequency of latent syphilis in women as compared with men. A statistical study of the admission diagnoses of 5410 patients from the Syphilis Department of this hospital appears in Table I. It is shown first, that secondary

TABLE I.

Admission Diagnoses of 5410 Syphilitic Patients Showing Sex Differences, particularly in Latent Syphilis and Neurosyphilis

| | Admission Diagnoses | Males | | | Females | | |
|---|---|---|---|---|---|---|---|
| | | Total Cases | Per cent of total males | | Total Cases | Per cent of total females | |
| Early | Primary | 314 | 10.56 | 36.64 | 41 | 1.68 | 24.77 |
| | Secondary early | 683 | 22.98 | | 523 | 21.45 | |
| | Secondary recurrent | 92 | 3.09 | | 40 | 1.64 | |
| Late (tertiary) | Tertiary skin & m.m. | 246 | 8.27 | 24.30 | 267 | 10.95 | 26.37 |
| | Bone | 209 | 7.03 | | 196 | 8.03 | |
| | Visceral | 109 | 3.66 | | 105 | 4.30 | |
| | Cardio-vascular | 158 | 5.31 | | 75 | 3.07 | |
| C.N.S. | All groups | 605 | | 20.35 | 158 | | 6.47 |
| Latent | Wassermann | 534 | 17.96 | 24.05 | 1085 | 44.50 | 47.57 |
| | History | 181 | 6.09 | | 75 | 3.07 | |
| Total | | 3131 | | | 2565 | | |
| Less duplications | | 286 | | | 127 | | |
| Total Cases | | 2972 | | | 2438 | | |

syphilis is equally frequent in the two sexes; second, no gross sex differences appear in the incidence of tertiary syphilis; third, neurosyphilis is three times as frequent in men as in women; and fourth, latent syphilis is almost twice as common in women as in men. The explanation of the milder course of the disease in females is, I believe, at least partially due to the influence of pregnancy. Of 1085 women with Wassermann positive latent syphilis, no less than 470, or 43.3 per cent, were pregnant at the time of the first examination.

*A Clinical Study of Syphilis in Pregnant Women.*—Two hundred women have been selected at random from the

* An incubation period of 54 days, in contrast to an average of 10 days in the controls. Seven of these eight animals were inoculated with syphilis and impregnated within a period of 24 hours, so that infection and impregnation practically coincided.

patients referred by the Obstetrical Department to the Syphilis Clinic, and from the non-pregnant mothers of syphilitic children. Of these, 44, or 22 per cent, had definite manifestations of syphilis, the remainder being free from gross evidences of the disease. For convenience in discussion, the patients have been classified as shown in Table II.

TABLE II.

Diagnostic Classification of 200 Women, in whom Syphilis was Complicated by Pregnancy

| DIAGNOSIS | |
|---|---|
| Syphilis primary and pregnancy | 3 |
| " secondary " " | 32 |
| " tertiary " " | 8 |
| " latent " " (primiparæ) | 63 |
| " " " " (multiparæ) | 72 |
| Non-pregnant mothers of syphilitic children | 22 |
| Total, | 200 |

*Early Syphilis and Pregnancy.*—A study of early syphilis complicated by pregnancy shows that certain women may react to syphilis contracted at the time of impregnation or during gestation with the usual manifestations of the infection, which are, however, much milder than when the disease is contracted independently of pregnancy. On the other hand, the normal time relations between primary and secondary syphilis may be disturbed, presumably by the factor of pregnancy; and the allergic condition predisposing to tertiarism may be reached with greater rapidity than is normally the case.

Thirty-five pregnant women had primary or secondary syphilis on admission. In three, all white primiparæ aged 19, 19, and 26, respectively, and each about 8 months pregnant, the lesion was a typical single chancre of 7, 10, and 14 days' duration, in which treponemes were demonstrated. Unless it is assumed that the incubation period of syphilis was altered by pregnancy, it is certain that infection occurred after the 6th month of pregnancy. No peculiarities were noted in the initial lesion.

Thirty-two of these patients, 9 white and 23 colored women, showed definite lesions of secondary syphilis on admission. All, except 5, were primiparæ and the average age was 19 years (white patients 21, colored 18). The majority were far advanced in pregnancy, the duration of gestation being 3 months or less in 6, from 3 to 6 months in 12, and from 6 to 9 months in 14 instances. In 9 instances a chancre was present in addition to the secondary lesions, and the date of infection could, therefore, be set at the time of or just before impregnation in 2 cases, in the second month of pregnancy once, in the third month twice, in the fourth month once, in the fifth month once, and in the sixth month twice.

In 23 patients with lesions of a secondary type, no chancre could be demonstrated. It is here more difficult to determine the date of infection, since it is not known if, and to what extent, the normal interval of 6 weeks between the primary and secondary periods has been distorted. In four patients, however, the secondary lesions had existed for a month or more before the date of impregnation. In four additional women, the dates of impregnation and of infection seemed about to coincide. In 7 patients, lesions appeared very late in pregnancy, on an average at the 8th month, and while no definite conclusions are permissible, it is likely that infection occurred after gestation had begun. In the remaining instances, the date of infection with relation to the pregnancy could not be determined.

It is important to note that the lesions of secondary syphilis were mild, as compared with those in non-pregnant females. Only 15 patients showed a generalized eruption, which was mild in 10, profuse in 5 instances. One negress had iritis. In 15 cases, the only lesions were moist papules or condylomata about the genitalia; in one instance, alopecia, and in another only papulo-erosive lesions of the mouth were present. The extent of the secondary outbreak bore no relation to the date of infection. Lesions were equally mild whether infection had taken place before or after impregnation. This is contrary to the usual text-book statement [20] that the lesions of early syphilis are more extensive and destructive during pregnancy than at any other time.

From the standpoint of the treatment of their own disease, as contrasted with the results to be obtained for the fetus, pregnant women are an unsatisfactory group to manage. So few are treated after the termination of their first pregnancy that a discussion of the results is fruitless. The results of treatment from the standpoint of the children has been thoroughly covered by Williams of the Obstetrical Department of this Hospital,[21] and need not be referred to here.

In the majority of these cases, no disturbance of the normal time relations of primary and secondary syphilis could be noted. That a distortion of the usual time intervals may occur is, however, exemplified by the following cases:—

Case I:—*Primary syphilis in ninth month of pregnaney. Secondary syphilis delayed for 9 months. Malignant secondary syphilis, recurrent. Infant, born healthy, developed a chanere on the lip at 20 months.*

A white woman, aged 26, developed a chancre of the vulva in the ninth month of pregnancy. At this time her husband presented papulo-erosive recurrent secondary syphilides on the penis. On admission, the chancre, in which treponemes were demonstrated, had been present for two weeks and the blood Wassermann reaction was positive. For reasons of her own, she was not treated at this time. The child was born normally and was a healthy baby. For 9 months following the appearance of the mother's chancre, no secondary lesions developed, in spite of the fact that she took no treatment. After this interval, however, she exploded with malignant rupial syphilis, consisting of large ulcerative lesions with heaped-up crusts, the largest lesion being 8 cm.

in diameter. After 5 doses of arsphenamin all lesions healed rapidly. She again disappeared from observation for one year, to re-appear when the baby was 20 months old. At this time she presented a recurrence of the same type as the first rash, including a large ulcerated lesion on the left nipple. The child, which she was still nursing occasionally, had just developed a characteristic initial lesion on the lower lip. The normal time interval of six weeks between primary lesion and secondary outbreak was, therefore, replaced by a lapse of ten months, approximately the duration of lactation. The secondary lesions, when they did appear, were exceedingly malignant in type.

Case II:—*Secondary syphilis and impregnation coincide. Irregular treatment. Ten and eighteen months later, respectively, precocious tertiarism.*

An analogous case was that of a white woman, aged 29. Her last menstrual period had been in August, 1919. In September, 1919, she developed a rash and a sore throat, which, together with a severe headache, persisted. On admission, January 21st, she was five months pregnant, and showed a generally distributed maculo-papular rash and mucous lesions of the mouth and genitalia. Infection, therefore, probably occurred just before impregnation. Between January and May, 1920, she received 10 doses of arsphenamin at irregular intervals, resulting in the rapid disappearance of lesions and the reversal of the blood Wassermann to negative by the 9th dose. Between May and August, 1920, she took no treatment because of the birth of her baby, which was born healthy at term. On August 3rd, 1920, about 10 months after infection, she returned with periostitis and osteomyelitis of the right radius and the left tibia, confirmed by roentgenoscopic examination. After 2 more injections of arsphenamin she once more disappeared, to return again 8 months later with a nodular syphilide of the chin and a gumma of the liver. Within the first one and one-half years of her infection, therefore, she had two clinical recurrences of a tertiary type and is to be regarded as an example of early allergy.

There are 9 additional patients of this group who have been followed for two to six years with repeated physical and serologic examinations. Of these, one has developed aortitis five years after infection. The others show no physical evidence of progression of the infection, in spite of very inefficient treatment. Two have subsequently borne four and five syphilitic children, respectively, in spite of their freedom from manifestations of the disease; one is Wassermann fast; and in another there have been frequent Wassermann recurrences. In four there have been no further physical, serologic, or obstetrical evidences of the disease.

In 8 of 11 cases, therefore, the infection has remained in a state of latency; and in spite of utterly inefficient treatment, four patients are clinically and serologically well on an average five years after infection.

*Primiparæ with Latent Syphilis.*—The patients with no obvious signs of syphilis may be divided into two main groups:—primiparæ in whom infection is presumably fairly recent, and multiparæ who, because of the previous birth of syphilitic children, may be regarded as having had syphilis for some years.

A review of the primiparous patients demonstrates that the infection is probably recent, because of the average age of the group, the histories obtained from several patients of only a few exposures, the development of a typical neuro-recurrence in one member of the group, and the response of the Wassermann reaction to treatment. It is evident that, if this be admitted, the simultaneous occurrence of infection and impregnation may result in a complete suppression of all early lesions of syphilis.

In this series are 63 primiparæ, of whom 7 were white and 56 colored. The average age was 21 (white average 20, colored 21), and 67 per cent of the patients were 21 years old or less. Twenty-four primiparæ, or 38.2 per cent of the group, gave positive or doubtful histories of infection.

In four patients there was a definite history of primary syphilis, which, in one instance, had been seen and diagnosed five and one-half years previously by another department of this Hospital. Seven told a detailed story of secondary syphilis, and in three of these the lesions had been recognized 9, 10 months, and 5 years, respectively, before admission. In 13 additional cases, there was a doubtful history which in 9 instances antedated pregnancy, and, in four, the suspicious lesions occurred during the first gestation. Thus, of the 24 patients with a positive or doubtful history, syphilis probably antedated pregnancy in 19. In no case did the dates of infection and impregnation seem to coincide. A history of treatment, other than a little mercury by mouth, was usually lacking.

In 39 cases, or 61.8 per cent of the primiparæ, no history of syphilis could be obtained. Twenty-one of these 39 women were single, and 14 had been married less than one year (7 within 6 months of admission). The average age of these symptomless patients was 19, two years less than the average for the whole group of primiparæ. One patient was only 15 years old, two were 16, six were 17, 8 were 18, and 5 were 19 years of age on admission. These facts are presumptive evidence that their sexual lives had begun fairly recently. The general rarity of contraceptive measures in this level of society leads one to believe that only a few contacts probably eventuated in impregnation. Two colored girls, aged 16 and 17, admitted only one and two sexual contacts, respectively, in each instance with only one man. In a white woman of 21, there was but one

TABLE III.

Showing the Type of Syphilitic Manifestations in Pregnant Women as Related to the Probable Date of Infection. (See text for discussion)

| Type of Syphilis | Total Cases | Infected long before pregnancy | Infected at about time of impregnation | Infected during Preg'cy | | | Uncertain |
|---|---|---|---|---|---|---|---|
| | | | | 1st-3rd month | 4th-6th month | 7th-9th month | |
| Primary | 3 | — | — | — | — | 3 | |
| Secondary early | 32 | 4 | 6 | 3 | 4 | — | 15 |
| Latent-primiparæ | 63 | | | | | | |
| History certain | 11 | 10 | — | — | 1 | — | |
| History doubtful | 13 | 9 | — | 2 | 2 | — | |
| History lacking | 39 | — | 39 | — | — | — | |
| Total | 98 | 23 | 45 | 5 | 7 | 3 | 15 |

intercourse, which resulted in impregnation. If it be admitted that the positive blood Wassermann found in these patients indicates that they are actually latent syphilitics, it is therefore not only possible, but highly probable, that infection occurred almost simultaneously with pregnancy in most of these 39 instances. The result was complete freedom from the lesions of primary and secondary syphilis.

The tabulation of the data from the physical and neurologic examination of these patients affords only presumptive evidence of syphilis, as in no instance were there definite lesions present. In 10 colored women were found pigmented scars on the body similar to those frequently seen in negroes, after secondary syphilis. In one instance, the scars were the definite remains of old annular lesions on the face. In 30 cases, or almost one-half, there was general glandular enlargement. Suspicious cardio-vascular abnormalities were detected in 2, (increased retrosternal dullness once, unduly accentuated and ringing second aortic sound once); in 7 instances there were inequalities, and irregularities, or a sluggish light reaction in the pupils, and in 7 additional cases pathologically increased deep reflexes.

If, as I have attempted to show, these primiparæ are actually recently infected with syphilis, the response of their blood Wassermanns to treatment ought to be similar to the type of response in early syphilis uncomplicated by pregnancy. In order to examine this question, composite Wassermann charts have been prepared by the following method:[22] The Wassermann results are given a numerical value, 4 corresponding to positive, 3 to suggestive positive, 2 to doubtful, 1 to suggestive negative and 0 to negative. The results before treatment and at the time of each injection of arsphenamin have then been recorded serially for each patient, a sample record being 444421. The serial results have been set down in columns, the columns added, and the total numerical value for each column divided by the number of cases in that column. A chart is thus obtained which expresses the average values for a fairly large series of cases. Only the results of the first course (six injections) of arsphenamin, representing a time period of from 6 to 9 weeks, have been utilized. The character and time intervals of later treatment are dependent on too many variable factors to make the results comparable. In Chart I the composite curve for the symptomless primiparæ is compared with similar results from a series of 70 unselected cases of secondary syphilis in males and females, in which pregnancy was not a complicating factor. The average values for the pregnant women are throughout somewhat lower than for the unselected group, but in general the curves closely parallel each other. This is presumptive evidence that the duration of infection in each group may be said to be approximately the same. Confirmation of

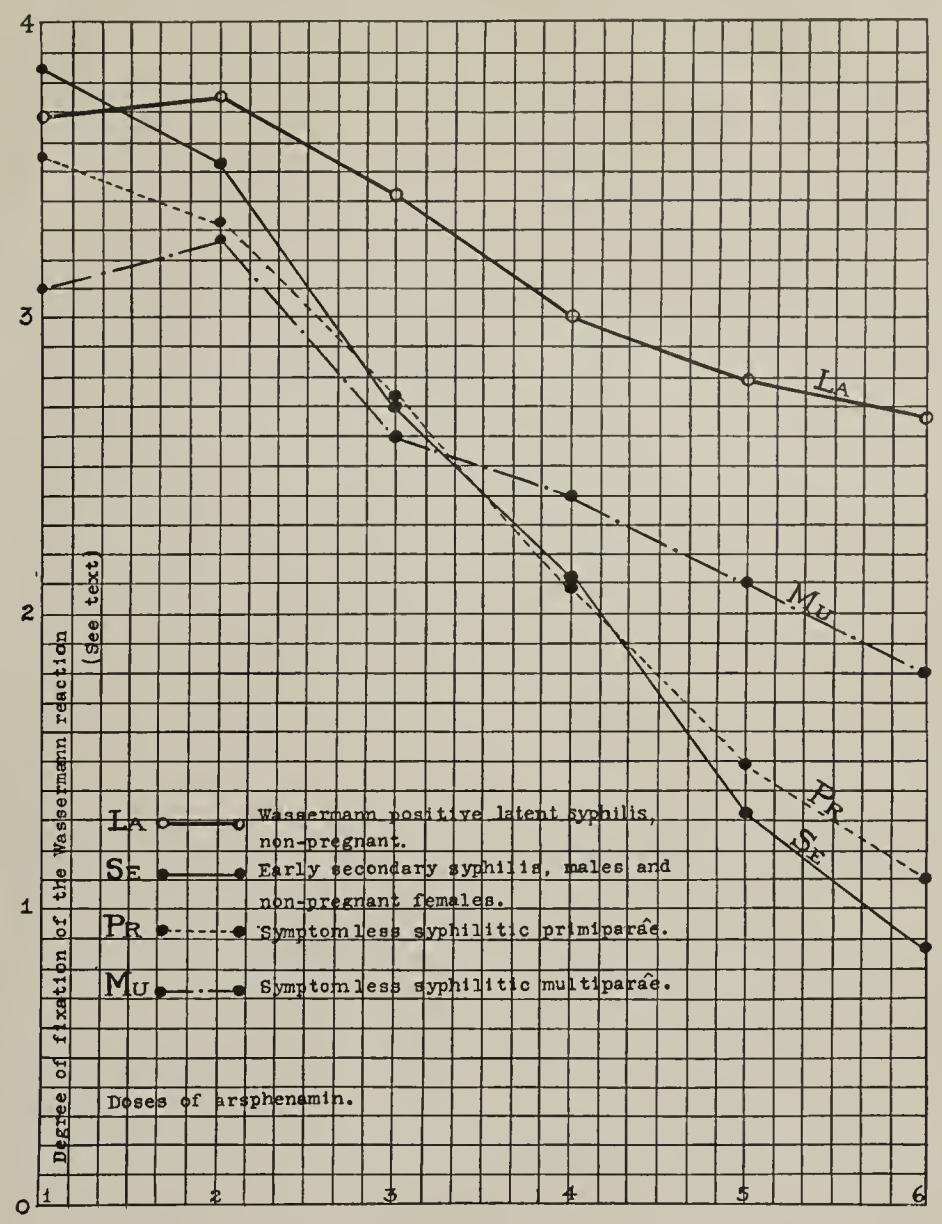


CHART I.

Showing the parallelism between the composite Wassermann curves (see text) of (1) symptomless syphilitic primiparæ and a series of unselected patients with early secondary syphilis; and (2)symptomless syphilitic multiparæ and a series of unselected non-pregnant patients with Wassermann positive latent syphilis. Note also the different types of response of primiparæ and multiparæ.

this hypothesis will be found in the discussion of the response to treatment of multiparæ, in whom the infection is of greater duration.

Eighteen primiparæ have been followed with repeated examinations for a period of from two to six years. One patient died with tuberculous peritonitis four months after delivery. Nine are clinically and serologically well, in spite of generally insufficient treatment. In 7 instances, 5 of which have occurred in Colles' women whose histories and physical examinations on admission were negative, there is definite evidence of progression of the syphilitic infection. Four have developed aortitis; one has a positive spinal fluid, but no symptoms of central nervous system syphilis; one has a probable syphilis of the liver. The 7th patient is of particular interest. Her history is briefly as follows:

Case III:—*Impregnation from a single contact. No history or physical evidence of primary or secondary syphilis. Wassermann positive. Treatment with arsphenamin; lapse; neuro-recurrence left N. viii; positive cerebrospinal fluid.*

A white girl, aged 21, unmarried, was admitted to the clinic 4 months pregnant on September 7, 1921. She denied syphilis and admitted only one sexual contact. Physical examination was completely negative. She received 6 doses of arsphenamin of 0.3 gm. each during the ensuing 2 months, the blood Wassermann remaining persistently positive. The pregnancy terminated by an abortion after the second injection. Following the first course of arsphenamin, the patient lapsed in treatment for 5 weeks. When she returned on December 12, 1921, a spinal puncture revealed 12 cells, globulin one plus, Wassermann positive with 1 c.c. of fluid, and negative colloidal gold and mastic curves. Before the puncture she had been completely symptomless. Immediately after, however, she was seized with a severe puncture headache lasting for two weeks and characteristically relieved by lying down. There then appeared a constant headache of different character, which was much worse at night. At the same time she developed marked vertigo and about 10 days later became deaf in her left ear. Otologic examination showed an inner ear deafness on the left with involvement of both vestibular and cochlear branches. In other words, there developed a typical neurorecurrence which was predicted by the results of her spinal fluid examination.

This type of neurosyphilis occurs only in patients with early syphilis who have lapsed following insufficient treatment, so that this case offers almost certain proof of a recent infection, the primary and secondary lesions of which were completely suppressed.

The question as to whether or not these pregnant women, routinely discovered to have positive blood Wassermann tests, are themselves syphilitic can be approached from the joint angles of history, physical examination, and subsequent course. Of the 63 primiparæ, the history or physical evidence or both were suggestive of syphilis in 46; while in 17 both were negative at the time of the first examination. In 7 of these 17 patients, however, the subsequent course made the diagnosis of syphilis certain (aortitis three times, positive spinal fluid once, neurorecurrence once, Wassermann fastness twice). In only 10 instances, 15 per cent, were all signs of infection (except a positive blood Wassermann) lacking. More exhaustive or prolonged study would probably provide additional evidence in these individuals.

*Multiparæ with Latent Syphilis.*—In the multiparæ, the anamnestic and physical evidence and the response of the blood Wassermann reaction to treatment lends weight to the opinion that all these mothers of syphilitic children are themselves probably syphilitic. In half the cases, there were no physical manifestations of the disease, in spite of the fact that the infection dated back from one to thirteen or more years; and in only eight patients (10 per cent) were outspoken lesions present. This group includes an additional patient whose course illustrates the distortion of the normal time relations of syphilis, with the early appearance of tertiarism.

There were 80 multiparous pregnant women referred because of a positive blood Wassermann. Of these 9 were white and 71 colored. The average age was 25, four years greater than in the primiparous group; 85 per cent of these women were over 21.

These women, together with 20 non-pregnant mothers of syphilitic infants (to be discussed presently) had had 319 previous pregnancies, the results of which are summed up in Table IV. Only brief mention of this point need be made, since many adequate similar studies are on record.[23] Forty-one per cent of the pregnancies resulted in mis-

TABLE IV.

Results of Pregnancies Occurring Before Admission or Treatment in 100 Syphilitic Women

| Mothers | Total pregnancies | Outcome Syphilis | | | | Outcome Unknown | | Living and well |
|---|---|---|---|---|---|---|---|---|
| | | Miscarriages | Still births | Died in infancy of syphilis | Living, syphilitic | Died in infancy; cause uncertain | Living but not examined | |
| 100 | 319 | 104 (32.6%) | 27 (8.4%) | 29 (9.0%) | 35 (10.9%) | 29 (9.0%) | 78 (24.4%) | 17 (5.3%) |
| | | 131 (41.0%) | | 64 (20.0%) | | | 95 (39.7%) | |
| | | 195 (61.1%) | | | | | | |
| | | 224 (70.2%) | | | | | | |

carriages or still-births, and 20.0 per cent in the birth of living syphilitic infants, almost half of which died during early infancy. It may be said with practical certainty that 61.1 per cent of all these pregnancies resulted in syphilitic children. In addition, there is an unknown incidence of syphilis represented in the 9 per cent of infantile deaths for which no definite cause could be assigned by the mothers, and in the 24.4 per cent of children who were living but not examined for syphilis. Only 5.3 per cent of the total pregnancies were definitely known to have produced normal children free from syphilis.

A positive or suggestive history of syphilis was obtained in 28 mothers, or 35 per cent. Four women had a definite history of a primary lesion; 14 gave convincing details regarding a secondary outbreak; and in 5 cases there had been various tertiary manifestations. In 5 additional cases, the history was doubtful, but might be construed as syphilitic. In the remaining 52 instances, or 65 per cent, no history whatever could be obtained.

There are available very few positive data regarding the date of infection. In three instances, the disease was contracted before any pregnancies had occurred. In two, infection took place late in pregnancy. In the remaining cases with a positive history infection had probably taken place before any pregnancies or in the intervals between them. In the patients with a negative history, the duration of the disease, based on the length of time since the earliest syphilitic birth, ranged from one to thirteen years.

The physical examinations of this group showed definite or suggestive signs of syphilis in 16 instances, or 20 per

cent. In 8, there were definite manifestations of tertiary syphilis consisting of bone lesions twice, ocular lesions twice, leukoplakia buccalis once, aortitis once, stricture of the rectum once, and gummatous adenitis once. In 24 of the 80 cases, there was well marked general glandular enlargement. There was in 5 patients some widening of the aortic arch, more than was consistent with the patient's age. Definite pupillary abnormalities were present in 5, and inequalities or sluggishness of the deep reflexes in 8 patients. In the remaining 42, physical examination was completely negative. A study of the interrelation of history and suggestive physical evidence shows, as might be expected, no correspondence. Sixteen patients who gave a positive or doubtful history showed one or more of the physical abnormalities enumerated. In 11, physical examination was completely negative in spite of a suggestive history. Of the 52 cases in which a history was absent, physical changes were present in 23 and absent in 29. In 51 patients, or 63 per cent, therefore, syphilis was suggested by the anamnestic or physical evidence or both.

Treatment in these patients, as in the other groups, so far as the results in the mother could be judged, was utterly inefficient. In order to examine further the thesis that in this group, as contrasted with the primiparæ, syphilis had existed for some years, a composite Wassermann chart for the first course of arsphenamin has been constructed. The comparison of this curve with the curve for primiparæ (Chart I) shows that the type of response is quite different. After the first injection of the drug a "provocative" curve is present in the multiparous, absent in the primiparous group; and the reaction in multiparæ is reduced toward negative much more slowly. In order further to illustrate this point, the composite Wassermann curve for multiparæ has been compared with that of an equal number of non-pregnant patients with Wassermann positive latent syphilis. The parallelism between these two curves, shown in Chart I, is quite striking.

Thirteen patients have been followed for a period of from 2 to 6 years. Of these, 9 have shown no signs of progression of their syphilis, whereas, in 4, repeated examinations gave evidence of progression either in the nervous system or in the cardio-vascular apparatus. Two additional patients are Wassermann fast.

In this group there is one patient whose history is analogous to those previously detailed.

Case IV:—*Primary lesion at 5th month of first pregnancy; secondary outbreak delayed for 12 months; epileptiform attacks (neurosyphilis) two years after infection.*

Two years before admission, a white woman, aged 23, when in the fifth month of her first pregnancy, had a genital sore about 2 cm. in diameter, the characteristics of which were those of an initial lesion. This sore persisted for eight months, or until four months after the birth of the child, which died at 6 weeks of congenital syphilis. No secondary symptoms appeared until 8 months after the termination of pregnancy, when she developed a generalized macular rash, marked alopecia and sore throat. These symptoms persisted for about 3 weeks and then disappeared spontaneously. Just before her admission more than a year later, she had a series of convulsions lasting practically all of one night. Physical examination at this time showed nothing except increased and unequal reflexes. Both the blood Wassermann reaction and examination of the spinal fluid gave positive results. It was later discovered that at the time of admission she was about one month pregnant for the second time.

Aside from this one instance, there were no striking deviations from the usual course of syphilis.

*Non-pregnant Mothers of Syphilitic Children.*—The fourth group is represented by 22 non-pregnant patients referred because of the recent or remote birth of a syphilitic child. In this group, there were 6 white and 16 colored women, 12 of whom were referred because of the discovery of syphilis in the placenta or fetus of a recent pregnancy, the remaining 10 because of their parentage of a syphilitic child ranging in age from 2 to 18 years. In 5 the child was more than 10 years old, so that, if the mothers may be presumed to have had syphilis, the disease was of considerable duration. From these 22 women, a history of primary syphilis was obtained once, of secondary syphilis twice, of a tertiary bone lesion once, and a doubtful history once, leaving 17 cases in which there was absolutely no history of the disease. The physical examinations of all except one were quite negative for gross signs of syphilis. There was general glandular enlargement in 6 cases. No evidence of cardio-vascular damage was found in any patient, though in 3 there were pupillary or reflex changes.

Case V:—*Mother of two juvenile parctic children is herself tabetic; history of syphilis absent.*

An interesting member of this group was a white woman, aged 48, who was referred because of the fact that her two eldest children, aged 18 and 16, had juvenile paresis, the youngest, aged 11, had congenital syphilis, while the third child, aged 13, was completely normal. Although this woman gave absolutely no history of syphilis, examination showed her to be typically tabetic and her blood and spinal fluid examinations were positive.

Case VI:—*Mother of a 9-year-old congenitally syphilitic girl; history, physical and neurologic examination, and repeated blood Wassermanns negative; cerebrospinal fluid paretic in type.*

Another patient, a white woman aged 37, whose husband was tabetic, was examined because her 9-year-old daughter had congenital syphilis. Her history, physical examination, and repeated blood Wassermanns were completely negative. She was about to be discharged either as a typical example of Colles' law, or as a spontaneous cure of latent syphilis, but a spinal puncture was fortunately performed. This revealed 104 cells, globulin four plus, Wassermann reaction positive with 0.1 c.c. of fluid, and paretic gold and mastic curves.

In striking contrast to these two patients are three women with syphilitic children aged 12, 16, and 17, respectively. In all of them, history, physical examination, ice-box Wassermann reactions, and spinal fluids were completely negative. This seems to demonstrate that a spontaneous cure of the disease is one of the possible results of the influence of pregnancy.

*The Wassermann Reaction in Pregnant Women.*—Attention has been drawn by a number of investigators, in particular Williams [21] and Menten,[24] to the fact that the blood Wassermann reaction in pregnant women is unreliable and that for diagnostic purposes it must be studied in connection with the findings in the placenta and fetus. Both of these observers lay stress on the fact that a positive Wassermann during pregnancy may spontaneously change to negative at the termination of gestation; others mention the occasional occurrence of a false positive reaction during the pregnant state.[25]

A study of the pregnant women reported in this paper leads me to believe that the unreliability of the Wassermann reaction in pregnancy consists in much more than the occasional spontaneous change of a positive test to negative after delivery. Among the group of 32 women with secondary syphilis, 3 had negative Wassermann reactions on admission, in spite of the presence of outspoken lesions of a secondary type. Among the 63 primiparæ, there were 7 instances in which the Wassermann reactions vacillated markedly from day to day, whereas similar phenomena were observed in 18 of the multiparous patients and in 3 of the women referred because of the history of a syphilitic child, making a total of 33 cases of 200 in which anomalous Wassermann results were observed. The change from positive to negative after delivery is not, in my experience, in any way characteristic, the reverse phenomenon of a negative Wassermann throughout pregnancy changing to positive shortly after the delivery of a syphilitic child having occurred in this series 5 times. In a number of other patients, the degree of fixation of the Wassermann reaction during pregnancy itself has been subject to striking variations. The results in 3 typical cases are shown in Table V. None of these women were treated during the period shown in the table.

TABLE V.

Illustrating the Vacillation of the Blood Wassermann in Pregnant Women

| Case | Date | Wassermanns | | | Duration of pregnancy at time of first Wassermann | Treatment |
|---|---|---|---|---|---|---|
| | | Water-bath | Ice-box | | | |
| | | Cholesterinized | Cholesterinized | Plain Alcoholic | | |
| F297 | 11- 9-21 | 2 | 4 | 4 | 4 mos. | none |
| | 12- 6-21 | 4 | 4 | 4 | | |
| | 12- 8-21 | 0 | 3 | 2 | | |
| | 12-10-21 | 4 | 4 | 4 | | |
| | 12-23-21 | 0 | — | — | | |
| F328 | 9-15-21 | 2 | 4 | 4 | 8 mos. | none |
| | 9-28-21 | 0 | 4 | 2 | | |
| | 10-11-21 | 1 | 4 | 4 | | |
| | 10-18-21 | 4 | 4 | 4 | | |
| F312 | 5-28-21 | 4 | 4 | 4 | 6 mos. | none |
| | 6-16-21 | 0 | — | — | | |
| | 7- 8-21 | 0 | 4 | 1 | | |
| | 7-20-21 | 4 | 4 | 4 | | |

In two instances, an explanation of this condition had apparently been discovered in an excess of native amboceptor in the blood of these women during pregnancy,[26] the excess disappearing within a short time after delivery. Laboratory investigations covering this point are at present being undertaken.

In discussing the reliability of the Wassermann during the pregnant state, mention should always be made of the technique employed. In the laboratory of this clinic, routine tests for diagnosis have been carried out by a test of only moderate delicacy and by water-bath fixation only.[27] In a number of cases falling into this general group, it has been noted that the ice-box Wassermann may be strongly positive when the water-bath test is negative.

*The Cerebrospinal Fluid in Pregnant Syphilitic Women.*—This question has been dealt with in a separate communication [28] and will not be entered into in detail at the present time. It was shown that, although invasion of the central nervous system takes place with equal frequency in the early stages of syphilis in males and in non-pregnant females, clinical neurosyphilis is, nevertheless, three times more frequent in men than in women. It was also shown that the incidence of abnormal spinal fluids in women with late syphilis was twice as high in a group of sterile women as in a similar group in which one or more pregnancies had occurred since infection. These findings and the negative evidence offered by the careful examination of the women in the present series indicate that not only may syphilis suppress the primary and secondary manifestations of the disease, but that the protection thus conferred, whatever its nature, may last over a long period of time and possibly for life. The majority of women who acquire syphilis simultaneously with the occurrence of pregnancy are, therefore, singularly free from the remote grave manifestations of the disease.

## Discussion

The observations of the pre-Wassermann era concerning the course of syphilis in pregnancy are in many respects lacking in scientific accuracy. Since 1905, the literature is largely concerned with the applicability of the Wassermann test to the validity of Colles' law, and the course of the infection in the pregnant woman has been more or less overlooked in favor of arguments for and against her status as syphilitic or non-syphilitic. In 1876, however,—forty-six years ago—Jonathan Hutchinson [28] reached the conclusion that all mothers of syphilitic children were themselves syphilitic and described the suppression of the symptoms of syphilis by pregnancy, and the distortion of the usual time relations of the disease, in much the same terms as those employed in the present study. The evidence now presented merely amplifies and lends weight to Hutchinson's conclusions and provides laboratory data inaccessible to him.

Hutchinson, and most of those who have followed him, regarded the unusual phenomena of syphilis in pregnancy to be due to the unusual mode of infection of the mother, that is, by blood stream infection from the fetus. The work of Trinchese has shown that this mode of infection is probably not possible; the animal experiments of Brown and Pearce and the clinical evidence afforded by the present study show that, even were paternal transmission possible, it would be unnecessary to employ it as an explanation for the silent course of maternal syphilis. The factor of pregnancy supplies some substance which is usually able to suppress the manifestations of the disease even when inoculation occurs by the usual route.

It is at present idle to speculate on the nature of this mechanism since no precise information is available. It is of course known that during the period of gestation, women are in general more resistant to chronic infections than at any other time; but in no disease other than syphilis does this protection extend over a period of years. It would be of great interest to have exact data concerning the behavior in pregnancy of other protozoan diseases closely allied to syphilis, such as framboesia and trypanosomiasis. Unfortunately, a fairly careful search of the literature yields no adequate information on this point.

Various hypotheses have been advanced to explain the protection afforded by pregnancy in syphilis. It may be that chemical alterations in the blood and tissues of pregnant women play some rôle. One outstanding alteration of the blood chemistry in pregnancy is a marked increase in the cholesterol content. This substance is not at all treponemicidal *in vitro,* it will not produce a positive Wassermann, and, in the blood at least, its normal content is unchanged in syphilis. The possible relationship of other chemical changes to syphilis is not at all clear.

Routh [30] has suggested that chorionic ferments cast into the maternal circulation may be the factor which suppresses the lesions of syphilis. This point, while interesting, has as yet received no scientific proof. The very existence of such ferments has not as yet been definitely confirmed.

The third striking difference between pregnant and non-pregnant women consists of definite changes in certain of the endocrine glands. The possibility that these changes might suppress the lesions of any infection is, however, remote.

The whole question of syphilis in relation to pregnancy demands careful restudy. The first step is the demonstration, if possible, that these Colles' women are actual carriers of *Treponema pallidum.* When this has been definitely proved, it will then be possible for the clinician, the chemist, the serologist, and the pathologist to attack together the larger question of the reason for the unusual latency of the infection.

## Summary and Conclusions

1. A critical study of the clinical and experimental evidence in the literature shows that in all probability neither Colles' law nor the theory of the paternal transmission of syphilis directly to the fetus are valid.

2. The clinical data supplied by this study of 178 pregnant women with positive blood Wassermann reactions and 22 non-pregnant mothers of syphilitic children supports this belief.

3. Forty-four of these women, or 22 per cent, had outspoken lesions of early or late syphilis at the time of admission. Of the remainder, syphilis was proved or strongly suggested by the history, physical examination, response to treatment, or subsequent course, or a combination of these factors, in 72 per cent. In only 21.5 per cent of the total 200 cases, therefore, were all evidences of syphilis (except a positive blood Wassermann) lacking.

4. This study also demonstrates that the factor of pregnancy may cause striking deviations from the usual course of syphilitic infection. If impregnation and infection approximately coincide, or if infection occurs during the course of pregnancy, the patient may develop the usual early manifestations of syphilis which are, however, much milder than if she is infected independently of pregnancy. Of those pregnant patients in whom the probable date of infection could be compared with the type of lesions present, approximately one-half behaved toward infection in this manner.

5. A slightly larger proportion of women, if infected with syphilis at about the time of impregnation, fail to develop any of the usual early lesions of syphilis. Under these circumstances, it is fair to assume that pregnancy is the factor which suppresses the lesions of the disease.

6. In a few patients (in this series, three of 200 women), the response to infection acquired at the beginning of or during pregnancy is markedly altered. The usual time relations between primary and secondary syphilis are much prolonged; on the other hand, the interval between early syphilis and tertiarism may be much shortened, and grave lesions of a tertiary type may appear early in the course of the disease.

7. The protection against the early lesions of syphilis afforded by pregnancy may persist over a long period of years, and possibly for a lifetime. Spontaneous cure of syphilis seems in a few instances to have been the ultimate result. In those women of this series who developed late syphilis, the viscera, and particularly the cardiovascular apparatus, were especially prone to involvement; whereas tertiary lesions of the skin or bones and neurosyphilis, either clinical or asymptomatic, were rare.

8. It is shown that in 33 of these 200 patients, the blood Wassermann reaction gave anomalous results. In 10 per cent of the pregnant women with secondary syphilis, the

reaction was negative. In the women with latent syphilis, it was prone to vacillate markedly without treatment; and in a number of cases, a negative or positive reaction during pregnancy spontaneously changed to the reverse after delivery. The possible factors responsible for this condition are briefly considered.

9. The nature of the mechanism by which pregnancy causes these alterations in the course of syphilitic infection is unknown. Various possibilities are mentioned.

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# SPINAL ARACHNOID GRANULATIONS WITH ESPECIAL REFERENCE TO THE CEREBROSPINAL FLUID

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*(From the Anatomical Laboratory of The Johns Hopkins University)*

In our modern conception of the circulation of the cerebrospinal fluid, the part played by the spinal nerve roots has never been conclusively defined. Anatomically, the arachnoid membrane has been generally accepted as being extended for a short distance along the emerging nerves and being reflected as the pia mater, thus forming a sort of cul-de-sac around the roots. But this cul-de-sac has not apparently been considered an anatomically or physiologically closed pocket, for cerebrospinal fluid has been supposed to escape at this point into the spaces surrounding the segmental nerves and be absorbed eventually by the lymphatics. The space lying between the nerve fasciculus and its fibrous sheath has been called most frequently the perineural space and has been assumed, thus, by many, to have a direct communication with the subarachnoid cavity and to act as a channel for part, at least, of the physiological drainage of the spinal fluid.

The existence of such a connection between the meningeal spaces of the cord and the peripheral nerves, if definitely proven, may also have a clinical interest. The transmission of the virus or toxin to the cord following peripheral infections, notably tetanus, may take place along such a pathway. On the other hand in various meningeal infections the escape of the virus from the cord into the nerves outside may occur by way of this channel.

In considering the possibility of such a fluid pathway, the importance of the study of the cord at the emergence of the spinal nerve roots must be apparent. Nevertheless, review * of the available literature reveals but few direct anatomical or physiological observations bearing on this problem. The studies have been made, in the main, on the peripheral nerves following the injection or introduction of a variety of materials into the subarachnoid space of animals and cadavers.

Perhaps the earliest observation of this kind was recorded in 1770 by Cotugno,[2] whom Garrison credits with the discovery of the cerebrospinal fluid. Cotugno injected mercury into the lumbar subarachnoid space of cadavers and demonstrated the liquid metal in the sciatic nerve. The nature of his material is open to adverse criticism; his procedure might, moreover, easily have ruptured the delicate arachnoid membrane.

In 1876 the classic work of the two Swedish anatomists, Key and Retzius,[7] appeared. Their large two-volume work on the central nervous system has been considered the basis of our modern conceptions of the course of the cerebrospinal fluid. Following subarachnoid injections of gelatin solutions containing Berlin blue, they found the blue color to extend outward along the spinal nerves for a considerable distance from the cord. They concluded, therefore, that definite pathways existed by which cerebrospinal fluid escaped from the cord into the substance of the nerves as well as into their perineural spaces. Upon critical examination of their evidence it appears, however, that such a conclusion is untenable, for their injections were made under excessive pressures (about 60 mm. Hg.), which certainly could have forced the gelatin into the peripheral nerves by mechanically entering the potential tissue spaces rather than passively following previously existing anatomical channels. The degree of pressure they used is evidenced in a sagittal section of the skull of one of their specimens. The cisterna cerebello-medullaris is markedly distended with the blue gelatin; the cerebellum is pushed upward and compressed, and the tentorium cerebelli is so deflected upward as to form an acute angle with the parietal cranium, compressing the occipital lobes before it.

Appearing before this work of Key and Retzius, but of a rather different nature, were the observations of Quincke [8] in 1872. During the course of subarachnoid injections of cinnabar, repeated in the living animal over several days, the spinal nerve roots were found to be always colored red. This extension was thought to indicate the existence of a pathway of absorption at this point. Quincke showed, however, that in this type of injection an intense inflammatory reaction was produced with the resulting phagocytosis of the irritating granules of mercuric sulphide. Such reactions under the influence of particulate matter have been studied recently by Essick,[4]

* A stimulating review of the literature on the cerebrospinal fluid was given in 1914 by Cushing.[3]

who found that the mesothelial cells lining the subarachnoid space formed active macrophages under these circumstances. The migration, therefore, of such granule-laden cells from the subarachnoid space can hardly be taken as evidence that any pathway for the escape of fluid exists normally.

In a similar way, in more recent years, Goldmann [6] injected solutions of trypan blue into the subarachnoid space and found the blue color in the spinal nerves at various distances from the cord. Like the cinnabar suspensions of Quincke, trypan blue produced an immediate, severe, toxic, and later inflammatory reaction, of which, indeed, the animals frequently died. Such a circumstance certainly renders less certain these observations as proof of the extension of the subarachnoid space into the peripheral nerves.

Flexner and Amoss [5] during the course of their studies on poliomyelitis injected a carmin granule suspension into the ventricles of monkeys and examined the animals after 24 hours. They found that the spinal nerve roots were always, and the posterior ganglia occasionally, colored red. Carmin granules, however, dissolve very readily, and it is very likely that instead of having traveled along existing channels as a granular suspension, the carmin had merely invaded the nerve roots as a soluble vital dye. No histological observations were reported.

The only direct study of the region at the emergence of the roots was made in 1904 by Sicard and Cestan.[9] They introduced India ink into the spinal subarachnoid space of animals, and examined the spinal nerve roots microscopically after eight to nine days. In this time, however, sufficient phagocytosis might have occurred to invalidate any definite conclusions in regard to the existence of channels connecting the subarachnoid space with the peripheral nerves. Nevertheless, even with this circumstance, they observed no passage of the black granules beyond the arachnoid membrane. Moreover, in cadavers, using strong syringe pressure, they could not observe any extension into the nerves following the injection of India ink into the lumbar space.

In spite of these observations, Cathelin,[1] in his monograph on the cerebrospinal fluid (1912) rather sweepingly ignores most of this evidence and graphically describes the cerebrospinal fluid as passing out along all the peripheral nerves to be absorbed by the lymphatics. He states, in fact, that "the peripheral nerves are bathed to their very termination by the same fluid as covers the central nervous system."

Similarly, one finds quite recently the statement by a German author, Weigeldt,[13] who considers the spinal portion of the subarachnoid space as absorbing the greatest part of the cerebrospinal fluid, that "at the emerging nerve roots one finds in all probability the most important point for the absorption of fluid."

One is left, therefore, with a mass of conflicting data, which, critically judged, certainly leaves one quite unconvinced that any communication between the subarachnoid space and the segmental nerves really exists. The problem becomes, briefly stated, an anatomical and physiological study of the spinal cord at the emergence of the roots: to determine the relations existing between the arachnoid membrane and the nerve roots, and between the subarachnoid space and the perineural space; and to determine whether any cerebrospinal fluid is absorbed at this point, and, if so, what pathway it follows.

## Methods

Of the various injection materials available, only a few have been used. In general, it was felt that for anatomical purposes a granular suspension was most suitable, while on the other hand, for physiological studies of the pathways of fluid absorption, the use of a true solution was to be desired. As indicated below, different methods of injection in different animals were used. The experiments performed may be grouped as follows:

1. Embryo pigs from 90 to 200 mm. in crown-rump length were injected with India ink through the left lateral ventricle under moderate and under strong syringe-pressure until the black ink was observed coming from the end of the lower spinal cord, cut across by severing the tail close to its base. The brain and spinal cord were then dissected out and India ink verified as passing from the ventricles to the subarachnoid space, and the presence of the ink particularly noted around the emerging roots. No microscopic studies of the material were made.

2. Two adult dogs were used. In one, 6 cc. of India ink were introduced into the cisterna cerebello-medullaris by occipito-atlantoid puncture, after an equal quantity of cerebrospinal fluid had been removed; the animal was killed immediately afterward. The other was killed with ether directly, no injection being made. The animals were then fixed by the injection of 10 per cent formalin through the aorta and the cord was dissected out. Blocks of the cord at the emergence of the roots were chosen and dehydrated in graded alcohols, starting at 50 per cent, with 10 per cent changes every 24 hours. After being embedded in celloidin, serial sections were cut, and were stained with aqueous carmalum solution.

3. Several dogs were used in this series. Cerebrospinal fluid (6 to 8 cc.), obtained from the cisterna cerebello-medullaris by occipito-atlantoid puncture, was replaced by an equal or smaller amount of a 1 per cent solution of equal parts of ammonium iron citrate and potassium ferrocyanide warmed to body temperature, the technique developed by Weed [10] being in general employed. The foreign solution thus introduced was later precipitated *in situ* by the addition of 1 per cent hydrochloric acid to the formalin injecting fixative.

The animals were anaesthetized with ether during these experiments. One animal was allowed to emerge from the anaesthetic for a short time to observe possible toxic effects. In this instance, aside from muscle twitchings and salivation, probably to be accounted for by the etherization, the animal showed no serious nervous symptoms. He walked normally, and, aside from moderate lethargy and stupor, showed no evidence of paralysis or other indications of disability.

The animals were killed at various periods from 15 minutes to one hour after the replacement, exsanguinated arterially and fixed by the injection of acidified formalin through the aorta, thus precipitating *in situ* the foreign solution, wherever it had made its way, as granules of Prussian blue. Serial sections were then prepared as described above.

It was early noted, when the time interval between replacement and final fixation exceeded one hour, that most of the blue color had disappeared and the presence of the dye could be demonstrated by the addition of acid only in the urine and saliva. Animals for microscopic study, therefore, were chosen from those in which the foreign solution had been allowed to remain only about 30 minutes, or in which replacement had been repeated 30 minutes before fixation.

4. Tissues from dogs prepared by Dr. L. H. Weed were used. These animals had received intravenous injections of hypertonic saline and the foreign ferrocyanide solution introduced under conditions of apparently increased absorption of cerebrospinal fluid. It was hoped, thus, to find a more striking demonstration of pathways of absorption from the cord, should they exist.

5. Cats which had received repeated injections of trypan blue intraspinally were also used. As the cells lining the subarachnoid cavity exhibit phagocytic activity (Essick), it was hoped to use this material to demonstrate, perhaps, the arrangement of the arachnoid cells at the emergence of the roots. All of this tissue on section, however, showed intense inflammatory reaction with an abundance of polymorphonuclear leucocytes especially in the pia mater, and was, therefore, not suitable for use in the present study.

## Experimental Findings and Discussion

From a study of the foregoing material it becomes possible to form a more definite conception of the region of the cord at the emergence of the spinal nerve roots. In drawing such a picture, however, it was found more advisable to gather together the experimental observations in different groups, depending on their application to the different anatomical and physiological problems discussed. A division under separate headings had been made, therefore, as indicated below.

*The perineural space.*—In all sections of the normal uninjected cord, as well as in all other tissue prepared by the usual histological methods, a distinct space was present about the nerve roots beyond their emergence from the cord, immediately beneath their fibrous sheath (Figs. 1 and 2). This was true for both the anterior and posterior divisions, except that around the spinal ganglia this space was absent, the sheath investing it tightly.

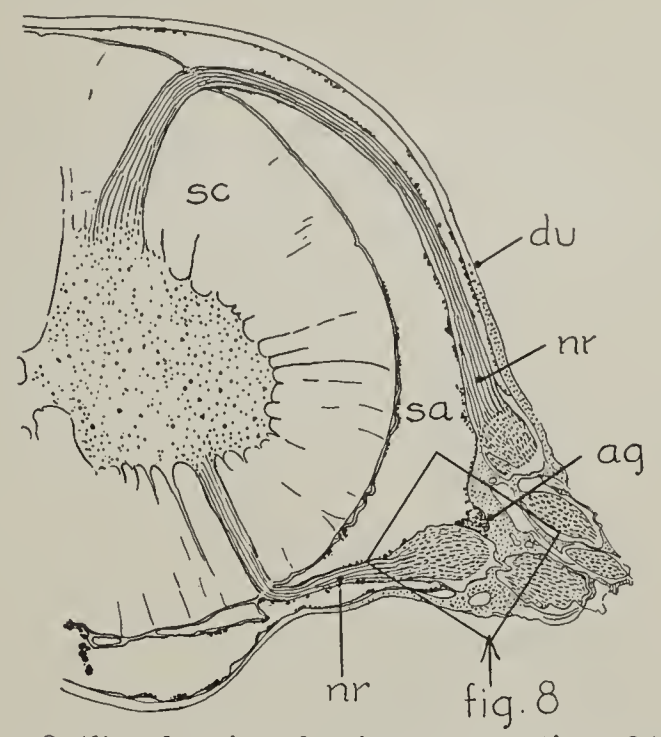


Fig. 1.—Outline drawing showing cross-section of the spinal cord of a dog, in which the subarachnoid space was injected, during life, with India ink. Fig. 8 shows a photomicrograph of the blocked-out area at higher power. (×10.)

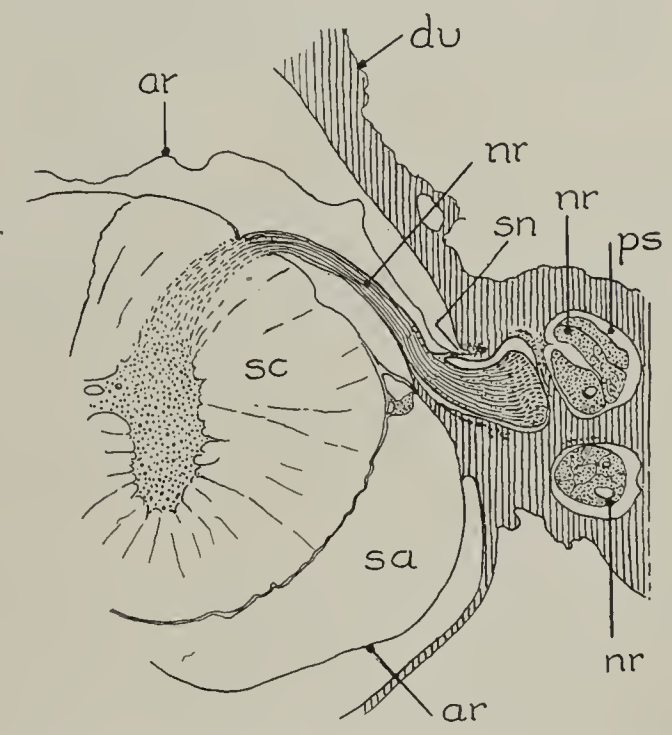


Fig. 2.—Outline drawing from cross-section of the cord in a dog, in which the Prussian blue reagents were introduced into the subarachnoid cavity replacing an equal quantity of cerebrospinal fluid, 30 minutes before death. The precipitated granules are shown extending outward from the subarachnoid angle (sn). The perineural space (pn) is shown surrounding each nerve root. Higher power photomicrographs of the subarachnoid angle are shown in Figs. 4 and 5. (×10.)

The size of this space was nearly uniform in its extent and was followed peripherally to the limits of the microscopic sections, a short distance beyond the posterior ganglia. Moreover, this space was limited on the one side by the smooth inner surface of the fibrous sheath and on

the other by the uncovered nerve fibers. Thus, at no point could any cellular lining be demonstrated. Though occasional loose strands of fibrous tissue were seen, no true trabeculæ were ever observed to cross this space. In no respect, therefore, did this space bear any histological resemblance to the mesothelial-lined subarachnoid cavity with its cell-covered trabeculæ.

Though this perineural space has been repeatedly observed, its existence in the living organism may be seriously questioned. Thus, one block of our series was dehydrated gradually and quickly over the course of 24 hours by immersion in an agitated solution of alcohol changed gradually from 50 per cent to absolute, with the use of an apparatus devised by Dr. C. H. Heuser of the Carnegie Institution of Embryology. This tissue, on section, showed no such space about the nerve roots, the fibrous sheath investing it tightly at all points (Fig. 3).

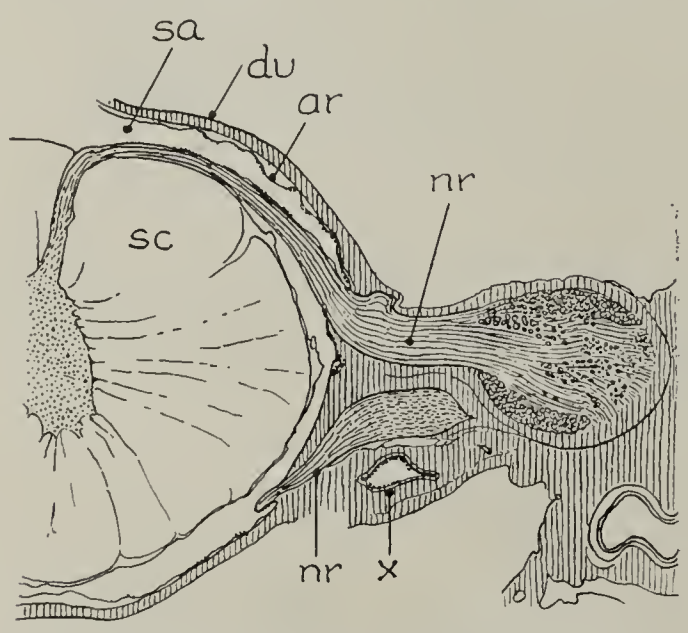


Fig. 3.—Outline drawing of a cross-section of the cord from a dog, in which the foreign solution was introduced following an intravenous injection of hypertonic saline solution. A large vein (X) shows an abundance of the Prussian blue granules within its lumen. This tissue was dehydrated gradually in 24 hours, and shows no perineural space around the nerve roots. Compare with Figs. 1 and 2, where a definite space is seen surrounding each nerve fasciculus, following the ordinary methods of dehydration. (×10.)

Whether this finding indicates that we are dealing merely with a tissue space, made apparent in the histological sections by the relatively unequal shrinking of the nervous and fibrous elements during the long dehydration and therefore an artifact, or whether it is truly an anatomical structure present in the living organism, cannot, as yet, be definitely said. More extended comparisons between the two methods of dehydration must be made.

*The Spinal Subarachnoid Angle.*—One finds in following the serial sections of both the injected and uninjected spinal cords that the arachnoid membrane bears a constant relation to the emerging roots. It does not really form a definite cul-de-sac, nor is it carried out with the emerging roots more than a millimeter or two, in the dog. It becomes immediately reflected back on the proximal side of the nerve root with which it blends and is carried back to become continuous with the pia mater. Thus, at this point of reflection the arachnoid forms an angle, which marks the limit of the subarachnoid space, at least as far as any space outside the cord is concerned. One can demonstrate, therefore, that, histologically, the subarachnoid space forms a closed cavity at this point and is not connected with any space in the nerve roots.

Moreover, in spite of repeated injections in embryo pigs, (90 to 200 mm. C. R.), rather strong syringe-pressure being used, in no instance could any extension of the India ink beyond the emergence of the spinal roots be found. The cervical lymph nodes were occasionally colored, but in no case did the spinal ganglia show any of the black ink on gross examination.

The study, finally, of the dog's cord which had been injected during life with India ink, bears out the same conclusion. At no point could the ink be demonstrated outside the subarachnoid space. The subarachnoid angles were outlined definitely with the black granules which nowhere penetrated the barrier of the arachnoid membrane (Fig. 8).

*The Spinal Arachnoid Granulations.*—Not only did the arachnoid membrane form an angle around the emerging roots, but at this point the cells of the arachnoid became more numerous, and formed definite cell-columns in some instances or veritable cell-clusters in others. In the microscopic sections these areas were easily recognized even under the low power by their deep-staining reaction as compared to the surrounding tissue (Figs. 6 and 7). Then, too, the character of most of these cells was rather different from the mesothelial cells covering the arachnoid membrane. Thus, their nuclei were more irregular, but more rounded, with well stained nuclear membrane but with little and pale-staining chromatin and one or two pale nucleoli, suggesting somewhat the vesicular character of epithelioid cells. The cytoplasm was indefinitely outlined and pale-staining. A few cells, however, were smaller with more densely and homogeneously staining nuclei resembling rather more the mesothelial cells of the arachnoid.

Though rather closely spaced, the distribution of these cells followed no definite pattern. In every instance, however, they blended with the contiguous dural prolongation, and in many instances actually invaded the fibrous substance of the dura. It can be seen, thus, how these cells would serve to close effectively the subdural space from connection with any space outside the cord.

The significance of these cells becomes more apparent when one considers that they are, in many ways, quite analogous to the arachnoid granulations in the cerebrum. In a recent paper Weed [11] has discussed the tendency of the arachnoid cells to proliferate at certain points, the adherence of these cells to the dura, their changes during senescence, their specialized property of permitting the passage of foreign solutions, and, finally, their possible

relation to the development of the so-called endotheliomata of the dura.

In the spinal cord, a quite comparable situation was found. These cells at the spinal subarachnoid angle exhibited the specialized property of permitting the passage through it of the foreign solution, while the remainder of the arachnoid membrane under the same circumstances remained impermeable. The size of these cell-clusters, moreover, varied in the different dogs, but since, unfortunately, no record of the approximate ages of these animals was kept, the age-relationship could not be traced. The adherence of these cells to the dura has already been noted. Finally, the relation of these cells to the occurrence in this region of spinal cord endotheliomata may, by analogy, be hypothesized.

*The Escape of the Foreign Solution Through the Spinal Arachnoid Angle.*—Injections of suspended material such as India ink show the anatomical limits of such a cavity as the subarachnoid space quite satisfactorily, but since the arachnoid mesothelium is impermeable to carbon particles, it is apparent that such injections cannot be used in tracing functional pathways of absorption, unless, of course, we take into account the possible existence of definite metapores or stomata, lymphatic channels, or the transport of particles by phagocytic cells. To investigate, therefore, the existence of physiological pathways of absorption for the cerebrospinal fluid a true solution must be used, capable of subsequent recognition in the tissues. The use of the mixture of iron ammonium citrate and potassium ferrocyanide, isotonic with body fluids and warmed to body temperature, as described above, was the method employed. The precipitation of these foreign salts *in situ* as granules of Prussian blue on fixation resulted in preparations showing the path that the introduced solution had followed.

Microscopic study of these specimens revealed the blue precipitate everywhere within the subarachnoid space, but none of the blood vessels within the cord or in the pia mater showed any of the colored granules within its lumen.

At the spinal subarachnoid angle, however, the solution, as evidenced by the blue precipitate, had penetrated into the cell-spaces of the arachnoid granulations, which, as had been pointed out, are adherent to and actually invade the dural prolongation at this point (Figs. 4 and 5). The granules were traced, thus, through the dura into the tissue surrounding the nerve roots outside the cord (Fig. 9). Whether the reagents finally entered lymphatic channels in this region could not be ascertained from the preparations made, but in some instances the blue color could be traced into the lumina of the large veins, which are quite numerous in this region (Fig. 3). The blue granules were never found inside the nerve fibers and were never observed in the so-called perineural space.

The pathway of escape of the Prussian blue reagents from the subarachnoid space, as above outlined, could be particularly well shown in the sections of the cord from animals in which the foreign solution had been introduced following the intravenous injection of hypertonic salt solution, which, as has been shown by Weed and McKibben,[12] causes apparently an increased outflow of fluid from the central nervous system (Figs. 3 and 9).

If these observations indicate that cerebrospinal fluid is actually absorbed at this point, it must at the same time be admitted that this pathway accounts for a relatively small percentage of the total absorption, under normal conditions, at least. In all of the animals examined after fixation, most of the blue precipitate, in the gross, was observed to have ascended upward to the base of the brain and over the cerebral convexities. Only a small percentage, relatively, had descended into the spinal subarachnoid space to be absorbed there.

## Conclusions

1. The arachnoid membrane in the spinal cord forms an angle as it is crossed by the emerging segmental nerve roots. This reflection of the membrane isolates the spinal subarachnoid space from anatomical connection with any space outside the cord. Suspended material injected intraspinally does not escape from the closed subarachnoid cavity at this point.

2. The space about the segmental nerve fasciculus, called the perineural space, was observed in all specimens prepared by the usual long dehydration methods. In one series dehydrated gradually in 24 hours this space was not apparent.

3. The cells of the arachnoid membrane proliferate at the spinal subarachnoid angle to form cell-clusters which are quite analogous to the cell tuftings of the arachnoid granulations in the cerebrum.

4. A foreign, non-toxic, isotonic solution, introduced into the subarachnoid space by the replacement of an equal quantity of cerebrospinal fluid, escaped from the cord through arachnoidal, mesothelial cell-nests and made its way into the veins outside the cord and possibly also into lymphatic channels in this region.

5. The existence of a fluid pathway physiologically connecting the subarachnoid cavity with the so-called perineural space could not be demonstrated.

This investigation was carried out with the most generous help of Dr. George B. Wislocki, for which sincere acknowledgment is made.

## KEY TO FIGURE REFERENCES

| | |
|---|---|
| ar | arachnoid membrane |
| ag | arachnoidal cell clusters |
| du | dura mater |
| nr | nerve root |
| pi | pia mater |
| pn | perineural space |
| sa | subarachnoid space |
| sd | subdural space |
| sc | spinal cord |
| sn | subarachnoid angle |

DESCRIPTION OF FIGURES ON PLATE 1

Fig. 4.—Photomicrograph of the subarachnoid angle in text fig. 2, showing the escape of the Prussian blue reagent, as indicated by the precipitated granules (shown in black), through the arachnoid cell-cluster into the substance of the dura. The perineural space shows none of these granules. (× 68.)

Fig. 5.—Photomicrograph of the region outlined in Fig. 4. (× 200.)

Fig. 6.—Photomicrograph of a cross-section of the normal uninjected spinal cord of an adult dog, showing the relationship existing between the nerve root and the subarachnoid angle. Extending from the subarachnoid angle and adherent to the overlying dura is seen the spinal arachnoid granulation (ag). (× 68.)

Fig. 7.—Photomicrograph of the region indicated in Fig. 6, showing in greater detail the character of the arachnoid cell-cluster. (× 300.)

Fig. 8.—Photomicrograph of the blocked area in text fig. 1, showing the distribution of the injected India ink granules, localized to and outlining exactly the limits of the subarachnoid space. A well-defined arachnoid cell-cluster is shown (ag). (× 65.)

Fig. 9.—Photomicrograph of a cross-section of the nerve roots of an injected dog, close to their emergence from the spinal cord, showing a portion of the subarachnoid space (sa), containing numerous particles of the Prussian blue. The perineural space is well defined, but shows none of the precipitated granules, which, however, are quite abundant in the surrounding dural tissue. (× 57.)

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# STUDIES OF THE BLOOD CHEMISTRY IN ALLERGY

By Ralph H. Major

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The importance of allergy as the underlying cause of many pathological conditions is now universally recognized. The subject has evoked a literature of enormous proportions and it has been studied from a great variety of standpoints. The great majority of these studies, however, have confined themselves to the immunologic and bacteriologic aspects of this problem, while investigations along metabolic lines have not received an equal attention. Investigations of the chemical problems involved in allergy may assist in unravelling its complex nature and in showing a common chemical basis for many somewhat diverse pathologic phenomena.

In a previous contribution [1] it was shown that there are marked disturbances in the nitrogen metabolism in allergy, with evidence of nitrogen destruction following anaphylactic shock. Hisanbu [2] noted increases in the amount of total non-protein nitrogen, urea and amino-nitrogen in the blood of guinea-pigs following anaphylactic shock. His method of investigation consisted in killing the animals that did not die in shock, pooling their blood and comparing the chemical findings with those of the pooled blood from normal guinea-pigs.

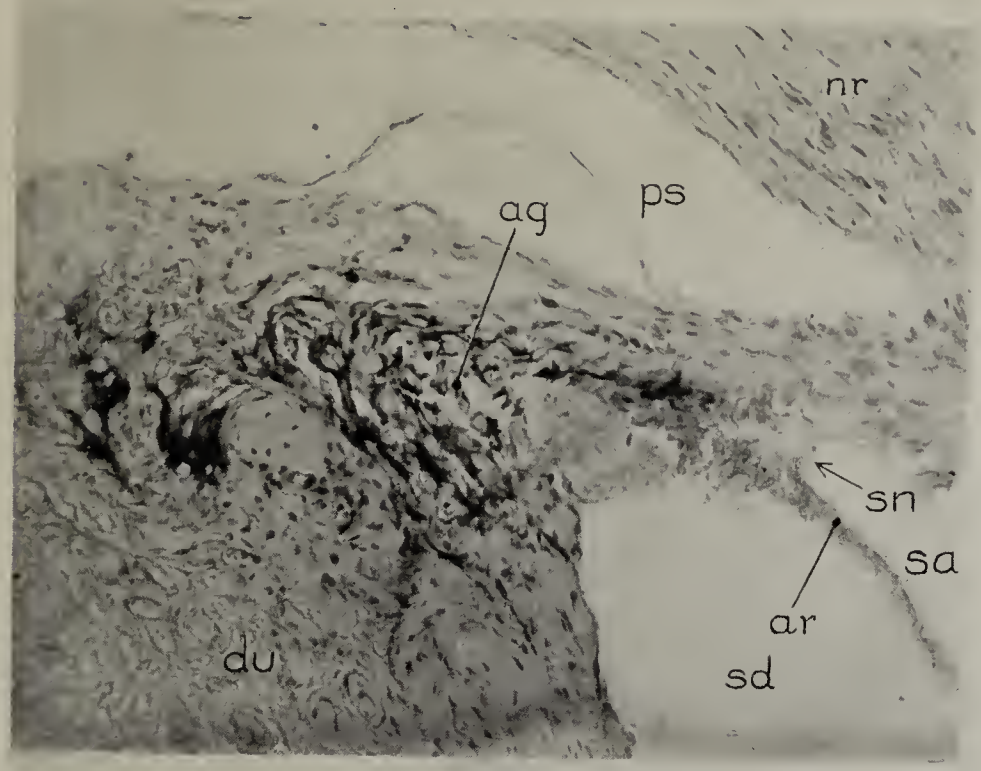
Rackemann, Longcope and Peters [3] studied ten cases of serum disease, occurring mostly during convalescence from pneumonia and noted often a marked but transient retention of chlorides and of water. Hirsch and Williams [4] have described a diminished alkalinity of the blood of guinea-pigs during anaphylactic shock, the change in reaction being at times so great as to be incompatible with life.

In my previous experiments it was found that rabbits, repeatedly injected with normal horse-serum, showed not only marked disturbances of nitrogen metabolism, but also lost weight rapidly, became very weak and soon died. Pentamelli [5] has described similar results following repeated intravenous injection of milk in rabbits.

All of these strongly suggest marked disturbances of metabolism in allergy and indicate the desirability of further investigations in the field.

ELMAN

PLATE 1

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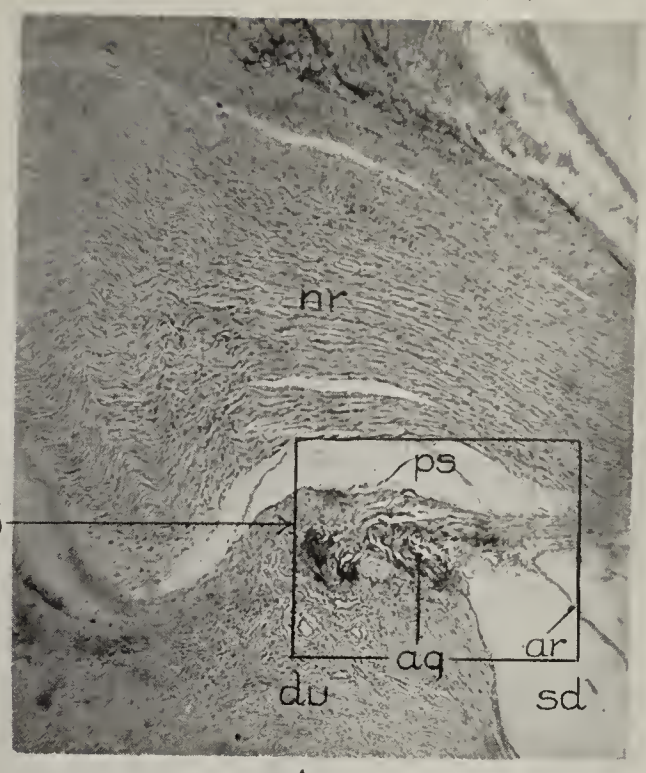


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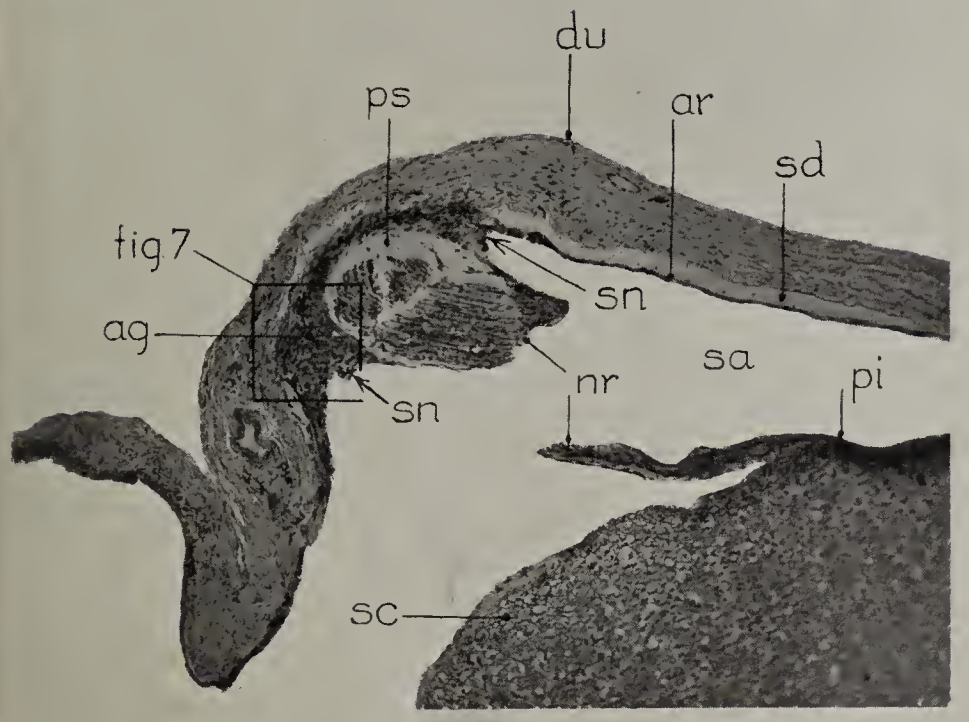


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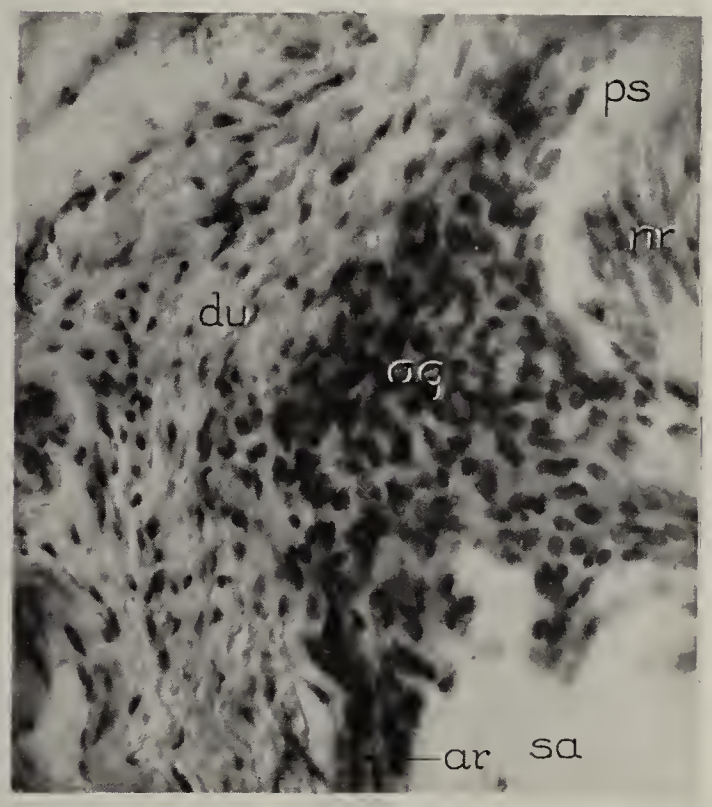


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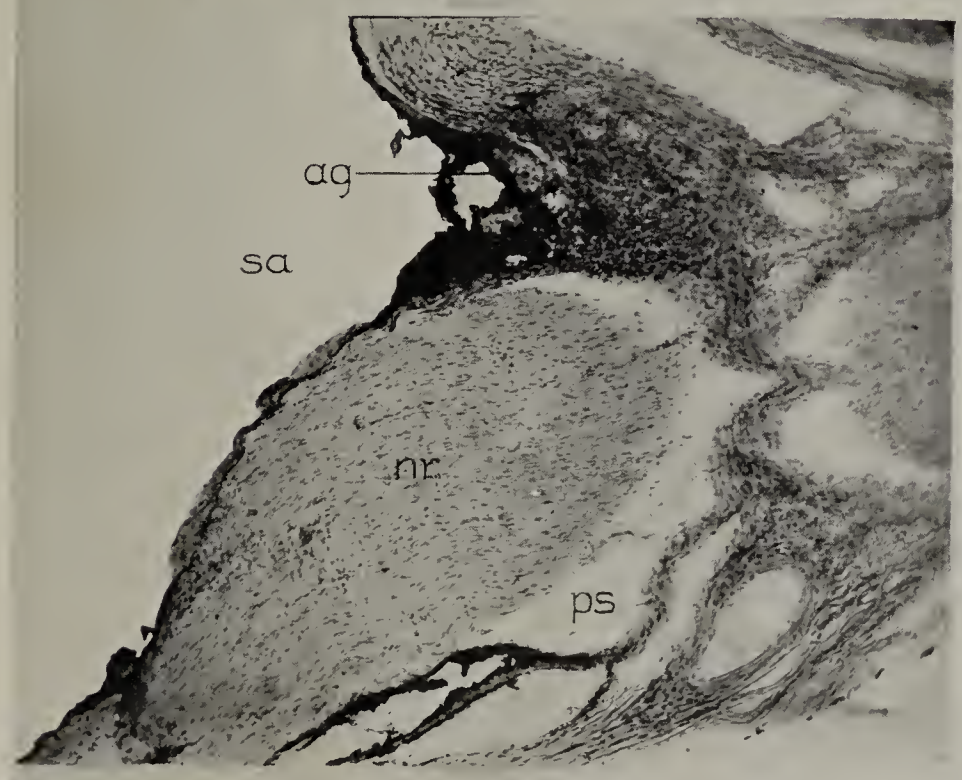


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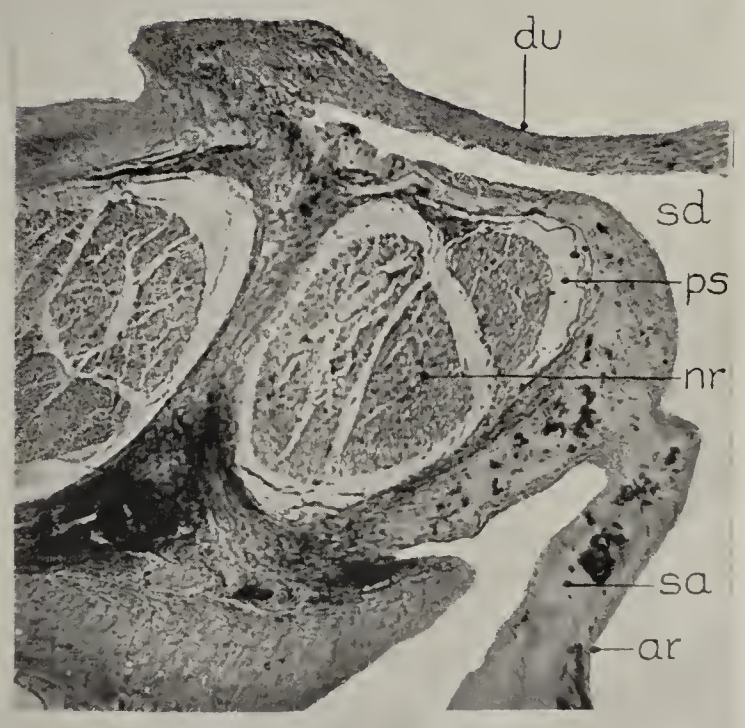


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In our experiments rabbits were employed, and horse-serum was administered to produce the allergic state. The animals were placed on three different diets, as described later, and before any experimental injections were made, a series of control animals were followed carefully in order to study variations in the blood and urine. Determinations of the blood non-protein nitrogen, urea, creatinine, chlorides and carbon-dioxide were made at frequent intervals before and after the intravenous injection of horse-serum. The urinary examinations included twenty-four hour estimations of the non-protein nitrogen, creatinine and chloride output.

### Methods

The non-protein nitrogen and creatinine determinations in the blood were carried out by the method of Folin and Wu. The blood urea was estimated by the method of Van Slyke and Cullen's modification of Marshall's method, blood chlorides were estimated on the tungstic acid filtrate after the technique suggested by Gettler, and the carbon-dioxide combining power of the plasma by the method of Van Slyke.

The urine chlorides were determined by the Modified Volhard-Arnold method, the total non-protein nitrogen by the micro method of Folin, and the creatinine by the method of Folin.

### Diets

Three different diets were employed. One series of animals was given a full diet consisting of oats, corn, bread, alfalfa hay and fresh vegetable greens. A second series was fed only bread and oats, while a third series received oats, bread and 1.0 gm. of acid sodium phosphate in capsules per day. For the sake of convenience these diets are called No. 1, No. 2 and No. 3.

### Controls

Fifteen animals on the three diets were observed over periods varying from two weeks to six weeks, with occasional blood examinations and daily urine studies.

No essential differences from the normal blood non-protein nitrogen, urea or creatinine were noted, the determinations showing variations in the non-protein nitrogen from 19 to 37 mg. per 100 c.c., blood urea 11 to 20 mg. per 100 c.c., the blood creatinine from 1.6 mg. to 2 mg. per 100 c.c.

No noteworthy differences were observed in the urinary non-protein nitrogen, chlorides or creatinine of animals on these three diets. The variations in the non-protein nitrogen excretion were from .07 gm. to 1 gm., the great majority of the determinations showing from .2 gm. to 8 gm. per day. Marked fluctuations in the chloride and creatinine output were noted. The excretion of chlorides varied from 0.04 gm. to 1.08 and the figures for creatinine excretion varied from 7 mg. to 206 mg. in twenty-four hours. The animals, when placed on oats and bread, and especially those receiving acid sodium phosphate, responded often with the production of an acid urine and in increased output of ammonia. This was not constant, however, and many animals continued to show an alkaline urine while on these diets.

The animals on Diet No. 1 gained weight and thrived, while those on Diet No. 2 and Diet No. 3 did not look so well and lost weight steadily, although not markedly. These latter two diets were apparently deficiency diets.

### The Effects of Injections at Frequent Intervals

Six animals received injections of 5 c.c. of horse-serum three days per week over a period of two months, there being only an interval of two days between the first and second injection. These animals never showed any symptoms of shock, they remained perfectly well during the experiment, frequent examinations of the blood showed normal values for non-protein nitrogen and urea, and the urine showed no abnormalities of excretion. The blood creatinine values following the second injection frequently were very high, varying from 3 mg. to 17 mg. per 100 c.c. Such high figures were not encountered in the control animals. Two of these animals were on a full diet, two were on Diet 2 and on Diet 3. The normal chemical findings show a parallelism with the picture of perfect health.

### The Effects of Injections at Longer Intervals

In this group of experiments the animals received a preliminary intravenous injection of 5 c.c. of horse-serum followed by a second injection in twelve or fourteen days and subsequent injections at intervals of eight to twelve days. Three animals received a full diet, three received only oats and bread, and six received oats, bread and acid sodium phosphate.

The animals in this series showed marked symptoms following injections, two died in shock, all were ill, and all but two on Diets 2 and 3 lost weight rapidly, became weak and finally died from one to two days after the last injection. Marked deviations from the normal blood chemistry were noted and also variations in urinary excretion, although the latter were not so striking.

*Total Non-protein Nitrogen.*—The total non-protein nitrogen of the blood, which was determined just before the injection and on the following day, showed a sudden rise, and this elevation has a tendency to be more marked in animals on the Diets 2 and 3 as the number of injections increased.

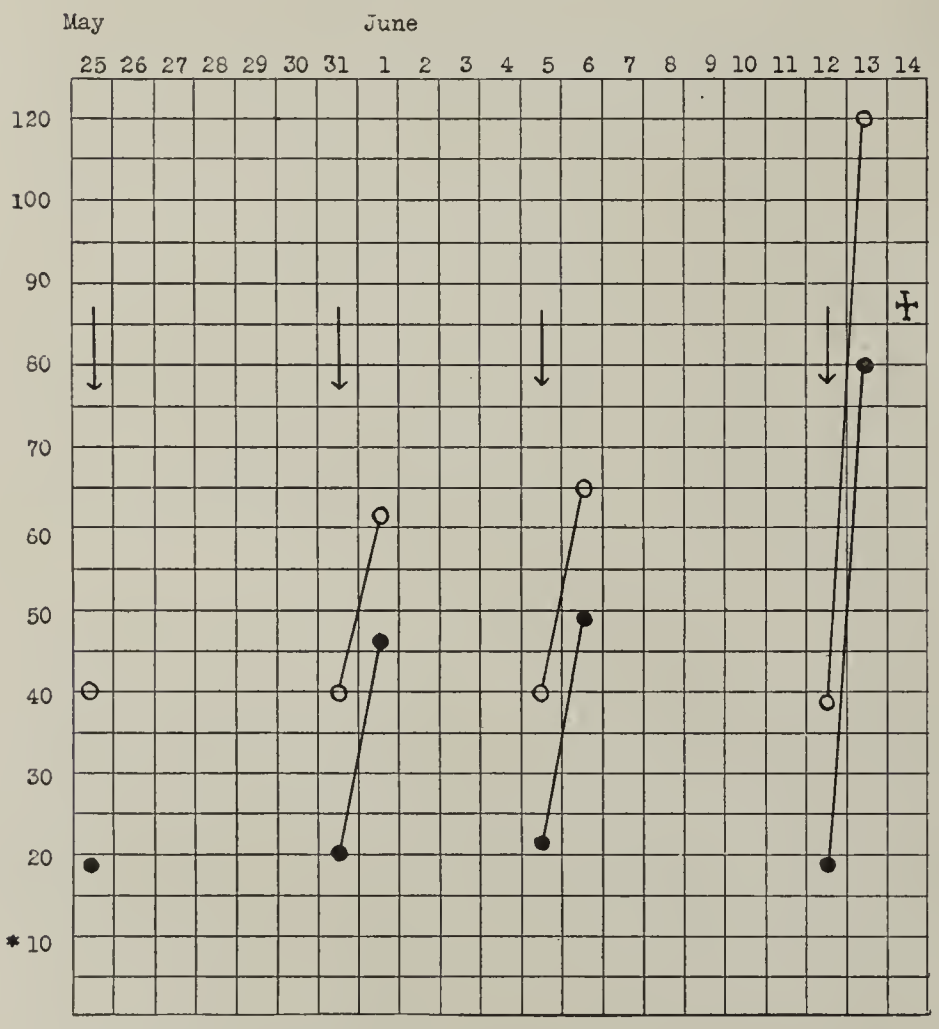
The animals on full diets frequently showed slight increase of non-protein nitrogen following injections, such changes, however, becoming less marked as the injections continued.

The animals on Diets 2 and 3 showed much more marked reactions. The readings before injections varied from 25

mg. to 38 mg. per 100 c.c., following injections values of 70 mg., 84 mg., 96 mg., 120 mg. and other high figures were obtained. One animal showed 57 mg. following the second injection, 62 mg. following the third injection and 96 mg. following the fourth injection. Another animal had a blood non-protein nitrogen of 41 mg. following the second injection, 62 mg. following the third injection, 66 mg. following the fourth injection and 120 mg. after the fifth injection, death occurring on this day (Chart I). The urine

CHART I.

Effects of Repeated Injections on the Blood Non-Protein Nitrogen and Urea




* Mg. per 100 c.c.
○ Non-protein Nitrogen.
● Urea.

frequently showed a diminution in the amount of non-protein nitrogen excretion during the first twenty-four hours after injection, followed by an increase during the next twenty-four hours.

*Urea.*—The animals on full diets showed elevations following the first three or four injections and then, as a rule, subsequent weekly injections produced no such effect. The readings before injections varied from 7 mg. to 16 mg. and following injections values of 15 to 35 mg. were obtained.

The animals on Diets 2 and 3 showed profound changes. The blood urea before injection varied from 12 to 20 mg. per 100 c.c., the average being 15 mg. On the day following the second injection there was invariably an increase in the blood urea. This increase showed a tendency to mount after each subsequent injection and, in animals which did not survive, values as high as 43 mg. and 60 mg. per 100 c.c. were encountered. One hour after injection the blood urea frequently fell to a figure lower than that before injection but on the following day it rose much higher than the previous level. One animal, for example, just before the second injection showed a blood urea of 27 mg. which fell to 22 mg. one hour after injection but rose the following day to 35 mg. per 100 c.c.

*Creatinine.*—The blood creatinine showed no noteworthy changes in animals on full diets. On Diets 2 and 3 it was increased in amount on the day after injection. Values of 6 mg., 5.9 mg. and 4 mg. were found.

The urinary excretion of creatinine following the intravenous injections showed as a rule a marked increase. The creatinine excretion, however, both in the control animals and in the treated animals during the periods between injections showed great variations, so that the results of the urine examinations were not so clear cut as those of the blood examinations.

*Carbon Dioxide.*—The carbon dioxide combining power of the blood plasma before and after injections of animals was studied. These determinations were made just before injection, one hour after injection and again on the following day. One hour after injection the carbon dioxide tension was generally lowered, but the following day it rose above the pre-injection level. This rise was very striking in some of the animals which were on Diets 2 and 3, one animal showing a rise from 12, before injection to 37 the day following. The fall in carbon dioxide one hour after injection was marked in some instances, declines from 28 to 8 and from 20 to 8 being observed.

These changes in carbon dioxide tension were most striking in the animals on Diets 2 and 3, but were also observed in animals on Diet 1 (full diet).

Hirsch and Williams have described marked lowering of alkalinity of the blood in guinea-pigs following anaphylactic shock. Our findings are in general harmony with theirs, although the fall is apparently transient and is followed by a marked rebound to a point above the original level.

*Chlorides.*—The blood chlorides showed little change in animals on Diet 1, while animals on Diets 2 and 3 showed a decided fall the day following injection. This change was very striking and quite as constant as the other marked chemical changes described. Such falls as from 620 to 580 mg., 600 to 500 mg., 560 to 460 mg., were observed. It is of interest to note that this fall in blood chlorides was accompanied by a rise in the carbon dioxide combining power of the serum, suggesting an intimate relationship between the two, indicating that perhaps the chlorides are concerning in increasing the alkalinity of the serum. Such a fall in blood chlorides was noted, as already mentioned, by Rackemann, Longcope and Peters in their studies of serum disease in man.

The studies of chloride excretion showed great variations from day to day. An increased excretion, however, was not noted following injections, while a decrease was often observed and complete suppression was seen once.

The urine on the day following injection frequently showed numerous hyaline casts and several animals on Diets 2 and 3 showed a marked hæmaturia persisting for from twenty-four to forty-eight hours.

## Discussion

Very marked differences in intensity of reaction characterize the results obtained in animals who are injected in a uniform manner, but who are on different diets. The rise in the blood non-protein nitrogen, urea and carbon dioxide on the day following injection was present in all animals injected at intervals of seven to ten days, but these changes were more marked in those on Diets 2 and 3. (Table 1). Animals on Diet 1 (full diet), after a few

TABLE I.

Blood Chemistry in Animals Injected at Intervals of 7 to 10 Days.

| Time | Date | Rabbit | Diet | N. P. N. | Urea N | Creatinine | Chlorides | $CO_2$ | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| Before Inj. | 6/19 | 42 | 3 | 26.8 | 9.8 | | 520 | 15 | Inj. No. 4 |
| 24 hrs. later | 6/20 | | | 84.4 | 28 | | 390 | 29 | Recovery |
| Before Inj. | 6/6 | 37 | 2 | 40 | 23 | 2.8 | | 10.6 | Inj. No. 3 |
| 24 hrs. later | 6/7 | | | 66 | 42.4 | 6.4 | | 22.6 | Recovery |
| Before Inj. | 5/17 | 13 | 3 | 37 | 18 | 1.7 | | | Inj. No. 5 |
| 24 hrs. later | 5/18 | | | 120 | 67.1 | 3.1 | | | Death |
| Before Inj. | 6/13 | 17 | 2 | 68 | | 2.5 | 630 | 8 | Inj. No. 3 |
| 24 hrs. later | 6/14 | | | 85 | | 3.8 | 570 | 20 | Recovery |

injections, recovered their equilibrium which was not further disturbed, while animals on Diets 2 and 3 showed as a rule more marked reactions with each injection and before death, which usually occurred, showed a high blood non-protein nitrogen, urea and creatinine. A very striking finding in this latter group was the fall of the blood chlorides.

At the beginning of these experiments animals were fed on oats and bread and on oats bread and acid sodium phosphate with the purpose of attempting to affect the alkali reserve. There was no evidence that any such effect was constantly achieved, although such changes were seen from time to time. Approximately one-half of the animals on these diets showed an acid urine. Studies of the effect of such diets on the carbon dioxide combining power of the serum gave similar inconstant results. Preliminary examination of the blood serum of a group of ten normal untreated animals showed values ranging from 23.5 to 36.2 volumes per cent (Table 2). Some of the animals on Diets 2 and 3 showed values at times as low as 7.3 and 5.3 in intervals following the second and third injections, although later the values were as high as in

TABLE II.

Carbon-Dioxide Combining Power of the Blood Plasma of Normal Rabbits.

| In Volumes | Per Cent | In Volumes | Per Cent |
|---|---|---|---|
| No. 1 | 36.2 | No. 6 | 26.8 |
| No. 2 | 27.2 | No. 7 | 29.6 |
| No. 3 | 25.4 | No. 8 | 30.5 |
| No. 4 | 34.3 | No. 9 | 29.6 |
| No. 5 | 36.2 | No. 10 | 23.5 |

the normal controls, in spite of the fact that these animals were showing marked reactions and later died. Two of the animals showing the highest figures for non-protein nitrogen, urea and creatinine in the blood had an alkaline urine throughout.

Diets 2 and 3 were deficient diets and it is highly probable that this deficiency played an important rôle in the results obtained.

The question of the rôle of the kidneys in the production of the significant changes in the blood chemistry is an interesting one. The animals after injection showed almost uniformly a high grade albuminuria with many casts and at times a marked hæmaturia. The urinary excretion of non-protein nitrogen was frequently markedly diminished the day following injection. During the periods when the animals showed very high values for blood non-protein nitrogen and urea, the urinary excretion of non-protein nitrogen was much lower than the average. All of these facts point to renal insufficiency. On the other hand, the increase in urinary creatinine and the diminished chloride excretion, accompanied often by a lowering of the blood chlorides, could not be explained on the basis of renal insufficiency. This makes it highly probable that we are dealing in these changes with both a renal and an extra-renal factor as suggested by Rackemann, Longcope and Peters in their studies on serum disease.

## Summary

The repeated injection of horse-serum into rabbits produces marked changes from the normal blood chemistry. These changes are more marked when the animal is on a deficiency diet.

The blood non-protein nitrogen, urea and creatinine are increased in the after anaphylactic shock, the blood chlorides are usually diminished. Urinary studies show there is usually a diminution in the nitrogen and chloride excretion and an increase in creatinine on the day following anaphylactic shock.

There is evidence that these changes are due both to a renal and an extra-renal factor.

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## ERRATUM

Attention is called to the fact that the plates in the January number of the Bulletin should have been numbered I, II, and III, instead of LXX, LXXI, LXXII.

# NOTES ON NEW BOOKS

*A Guide to Urinary Diseases.* By Adolphe Abrahams, O.B.E., M.D., M.R.C.P. and A. Clifford Morson, O.B.E., F.R.C.S. (*New York, Longmans, Green & Co. London, Edward Arnold & Co., 1921*).

This book is addressed mainly to the general practitioner and fourth year student. As outlined in the preface, its object primarily is to acquaint the reader with the symptoms of urinary diseases and thus assist him in identifying the various lesions.

Pathology is very superficially considered and only the simpler therapeutic measures are dealt with. The book contains little or nothing of value which is not included in modern text-books on urology. W. A. F.

*Clinical Diagnosis.* By Charles Phillips Emerson, A.B.M.D. $7.50. (*Phila. & Lond., J. B. Lippincott Company, 1921.*)

This is the 5th edition of Emerson's Clinical Diagnosis. The book is larger and fuller than its predecessors, and is markedly superior in many respects. All subjects in Clinical Diagnosis are taken up in considerable length, and with great completeness. Of the 709 pages in the work, 87 are devoted to the sputum, 250 to the urine, 87 to gastro-intestinal contents and feces, 249 to the blood, 14 to the cerebrospinal fluid and 16 to the various other body fluids. The methods used are those that have stood repeated trials in the clinical laboratory of the Johns Hopkins Hospital and Indiana University Medical School. These are described with care and often at considerable length, so that those who have not had a thorough laboratory training may understand their value and significance. It is an excellent book for medical students, laboratory workers and practitioners of medicine. Dr. Emerson is to be congratulated for the thoroughness and completeness with which this 5th edition has been brought up to date and rendered available. J. G. H.

*Ophthalmoscopy, Retinoscopy, and Refraction.* By W. A. Fisher, M.D. (*Chicago, W. A. Fisher, 1922.*)

An elementary treatise on ophthalmoscopy, the examination of the eyes and refraction, intended for the general practitioner and medical students. In so far as the book may stimulate the practitioner to include an examination of the eyes in his general physical examination, and call attention to refraction errors, this treatise may possibly be of some value. As a work on ophthalmology and refraction, it is too elementary to supply the needs of medical students, unless supplemented by a real text-book on ophthalmology. Serious exception might be taken to the brief presentation of some of the subjects. A. C. W.

*The Treatment of Fractures.* Charles Locke Scudder, M.D. (*Phila. & London, W. B. Saunders Co., 1922.*) $8.50.

The ninth edition of "The Treatment of Fractures" by Scudder is unquestionably one of our best English books on this subject. The author has succeeded in producing a book which is sufficiently simple to serve as an excellent text-book for students and yet one which is sufficiently full and up-to-date to be a valuable reference book for practitioners. One is impressed with the relatively large proportion of the text devoted to the treatment of fractures and the elimination of lengthy descriptions of their varieties and causes. If any single element of the book deserves particular praise, it is the illustrations. These alone make the volume well worth possessing.

On the whole the principles advocated by the author are the accepted ones. There are, however, a few points on which one might differ. For instance, fractures of the scaphoid bone require considerably longer immobolization than three weeks. It might be suggested also that the author's periods of immobility for fractures about the elbow are on the average one week too long. The statement that fractures of the neck or head of the radius are not common is not verified by all statistics. Furthermore, the treatment of this particular group of fractures certainly requires more than a single sentence. In places the arrangement of the text is unsatisfactory. For example, the general discussion of the subject of non-union should not be treated as a subdivision of the progress of fractures about the elbow, but should constitute a separate chapter. Similarly, the paragraph on the selection of apparatus or splints should not be interpolated in the chapter on fractures of the elbow but might form a separate chapter and should be treated in much greater detail. The subject of the treatment of compound fractures is too brief. A paragraph on injuries to the coccyx might well be inserted in the chapter on fractures of the pelvis.

It is gratifying to note the stress which the writer places upon massage and passive motion, but in no place in the text is the fact emphasized that the treatment of every fracture is an individual problem. Furthermore, a sharper distinction should be drawn between a Colles' and a Barton's fracture, since the same treatment does not hold for both. It is now generally admitted that better results are obtained from the use of plaster splints than from the use of those made of board, and although the chapter on the use of plaster is good, the author does not emphasize throughout his work the advantages of this type of splints. W. M. F.

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